Effect of the ear canal occlusion on pure tone thresholds and its clinical applicability in validation of the contralateral occlusion test

LUÍS MIGUEL ROQUE DOS REIS

Thesis submitted for the degree of Doctor in Medicine - Health of Populations in the Specialty of Clinical Research

Faculdade de Ciências Médicas | NOVA Medical School, Universidade NOVA de Lisboa

July, 2020

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In memory of my father and mother,

for their immeasurable support.

To my loved ones, Carolina and Miguel.

“Things are what they seem to be;

Or they are and do not seem to be;

Or they are not, but they seem to be;

Either they are not, nor they seem to be.”

Epictetus (53–130 b.C.)

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PUBLICATIONS DURING CANDIDATURE

This study allowed several publications included in the thesis. For each publication, I was the leading responsible for the original idea, review of literature, research plan and design, recruitment, data collection, analysis and interpretation of results and writing and reviewing of the manuscript.

Publications related to the thesis

Reis LR, Fernandes PV, Escada P. Contralateral Occlusion Test (COT): The effect of external ear canal occlusion in hearing thresholds. Acta Otorrinolaringol Esp. 2017 Jul - Aug;68(4):197-203. doi: 10.1016/j.otorri.2016.11.011. Epub 2017 Feb 10.

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Oral presentations related to the thesis

Reis LR, Castelhano L, Correia F, Escada P. Accuracy of Contralateral Occlusion Test in Conductive Hearing Loss. 14th European Federation Audiology Societies (EFAS) Congress. Lisbon, 22 to 25 May 2019.

Fernandes PV, Reis LR, Escada P. O efeito da oclusão do canal auditivo externo nos limiares auditivos. 63th Congresso da Sociedade Portuguesa de Otorrinolaringologia e Cirurgia Cérvico-Facial (SPORL-CCF). Coimbra, 21 to 24 April 2016.

Castelhano L, Reis LR, Correia F, Escada P. Teste de Oclusão Contralateral. O efeito da oclusão do canal auditivo externo com a idade. 65th Congresso da SPORL-CCF. Aveiro, 5 to 6 May 2018.

Castelhano L, Reis LR, Correia F, Escada P. Teste de oclusão contralateral (TOC): prever o grau de surdez de condução pelo efeito da oclusão do canal auditivo externo. 66th Congresso da SPORL-CCF. Peniche, 3 to 5 May 2019.

Awarded presentations

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Donato M, Reis LR, Sousa R, Escada P. Translation, Cultural Adaptation and Validation of the Satisfaction with Amplification in Daily Life Scale for European Portuguese. 63th Congresso da SPORL-CCF. Coimbra, 21 to 24 April 2016. First Prize for Communication in Otology, Otoneurology and Surgery of the Skull Base.

Castelhano L, Pimentel J, Reis LR, Lameiras R, Almeida G, Escada P. Vigilância pós-operatória de pacientes submetidos a mastoidectomia técnica fechada por colesteatoma. 64th Congresso da SPORL-CCF. Viana do Castelo, 5 to 7 May 2017. Second Prize for Poster in Otology, Otoneurology and Surgery of the Skull Base.

Castelhano L, Reis LR, Correia F, Escada P. Contralateral Occlusion Test (COT): The Effect of External Ear Canal Occlusion with Aging. 65th Congresso da SPORL-CCF. Aveiro, 5 to 6 May 2018. Third Prize for Communication in Otology, Otoneurology and Surgery of the Skull Base.

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ABSTRACT

Introduction and goals: Bedside testing may accelerate clinical decision by making a quick qualitative assessment of hearing loss. This study described the design and validation of a bedside test with tuning forks that allow some quantitative evaluation of hearing loss in the presence of unilateral conductive hearing loss. Three distinct phases were considered: the first phase of the study quantified the effects of complete external auditory canal occlusion on hearing, in order to decide which tuning fork frequency is more appropriate to use for quantifying the hearing loss with the novel contralateral occlusion test (COT). The reproducibility of occlusion between examiners was also explored in this phase of the study. The second phase of the study evaluated the effects of external auditory canal occlusion on hearing thresholds with aging, in order to decide which tuning fork is more appropriate to use for the COT in individuals of different ages. The third phase of this study evaluated the accuracy of the test in predicting the degree of hearing loss.

Methods: Over the study period, the three distinct phases considered population numbers of different age/size classes. At the first phase, 20 normal-hearing adults (40 ears) between 21-30 years old underwent sound field pure tone audiometry with and without ear canal occlusion. Each ear was tested with the standard frequencies (250, 500, 1000, and 2000 Hz) with warble tones. The contralateral ear was suppressed with the use of masking. Ear occlusion was performed by two examiners. In the second phase, 42 normal hearing subjects (84 ears) between 21 and 67 years were divided into three age groups (20–30 years, 40–50 years, and 60–70 years). Participants underwent sound field audiometry tests as described to the first phase of the study. In the third phase, 53 subjects with unilateral conductive hearing loss were recruited from an otolaryngology department

of a tertiary hospital. The COT was performed to determine lateralization using 128, 256, 512, 1024 and 2048 Hz tuning forks with the non-affected ear meatus totally occluded. Pure-tone audiometry was then performed, separately and blinded of the tuning fork test results, to establish the presence and degree of the air-bone gap (ABG) and the pure-tone average (PTA). The tuning fork responses were finally correlated with the ABG and the PTA to determine their accuracy in quantifying the degree of hearing loss.

Results: In the first phase of the study, occlusion of the external auditory canal determined an increase in hearing thresholds with increasing frequencies, from 19.94 dB (250 Hz) to 39.25 dB (2000 Hz). The difference on hearing thresholds between occluded and unoccluded conditions was statistically significant and increased from 10.69 dB (250 Hz) to 32.12 dB (2000 Hz). There were no statistically significant differences according to gender or between examiners. The second phase of the study demonstrated that hearing thresholds increased with higher frequencies from 20.85 dB (250 Hz, 20–30 years group) to 48 dB (2000 Hz, 60–70 years group). The difference on hearing thresholds between occluded and unoccluded conditions were statistically significant and increased, ranging from 11.1 dB (250 Hz, 20–30 years group) to 32 dB (2000 Hz, 20–30 years group). Statistically significant differences were found for the three age groups and for all evaluations except to 500 Hz difference and average difference. The mean hearing loss produced by occlusion at 500 Hz was approximately 19 dB. No statistically significant differences were found between right and left ears and gender for all measurements. In the third phase of the study COT showed a stronger association between hearing loss and the lateralization response using the 512 Hz tuning fork ($p = 0.001$). The sensitivity of the 512 Hz fork in detecting a PTA equal or greater than 35.6 dB was 94.6% and the specificity was 75.0% for a positive predictive value of 89.7% and a negative predictive value of 85.7%, assuming a pretest prevalence of 69.8%.

Conclusions: The occlusion method as performed demonstrated reproducibility between examiners and with aging. The occlusion effect increased the hearing thresholds and became more evident with higher frequencies. The COT was accurate in predicting the degree of unilateral conductive hearing loss. If lateralization to the affected ear occurred, it was almost certain that the affected ear has a moderate or severe conductive hearing loss. 256 Hz or 512 Hz tuning forks were the more appropriate for diagnosis of mild hearing loss, and the 2048 Hz tuning fork was the more appropriate for moderate hearing loss; but aging and accuracy studies demonstrated that the use of the 512 Hz tuning fork is the most suitable for COT. The use of this test may allow clinicians to distinguish mild from moderate or greater than moderate unilateral conductive hearing loss.

Keywords: Hearing loss, conductive; audiometry; ear canal; occlusion; hearing tests; bedside testing; auditory threshold.

RESUMO

Introdução e objetivos: Os testes de cabeceira podem diminuir o tempo de resposta clínica, melhorar a tomada de decisões e permitir uma rápida avaliação qualitativa da perda auditiva. Este estudo descreve o desenho e a validação dum novo teste de cabeceira com diapasão que permite quantificar a perda auditiva, na presença de surdez de condução unilateral. O estudo considerou três fases distintas: a primeira fase avaliou os efeitos da oclusão completa do canal auditivo externo sobre a audição, a fim de decidir qual a frequência de diapasão mais adequada para quantificar a hipoacusia com o teste de oclusão contralateral (TOC). A reprodutibilidade do método de oclusão entre examinadores também foi estudada nesta fase. A segunda fase avaliou o efeito da oclusão nos limiares auditivos de acordo com a idade, em indivíduos de idades distintas, para apurar qual o diapasão mais adequado na realização do TOC. A terceira fase avaliou a precisão do teste na previsão do grau de perda auditiva.

Métodos: Nas três fases distintas do estudo consideraram-se populações com idade e tamanho da amostra diferentes. Na primeira fase do estudo, 20 adultos (40 ouvidos) com audição normal (21-30 anos de idade) foram submetidos a uma audiometria tonal em campo livre, com e sem oclusão do canal auditivo externo. Cada ouvido foi testado com as frequências standard (250, 500, 1000, e 2000 Hz) em tons de warble. O ouvido contralateral foi suprimido por mascaramento. A oclusão do ouvido foi realizada independentemente por 2 examinadores. Na segunda fase, 42 adultos (84 ouvidos) com audição normal (21-67 anos de idade) foram divididos em três grupos etários: 20-30, 40-50, e 60-70 anos. Os participantes foram avaliados por audiometria tonal em campo livre, nas mesmas condições descritas para a primeira fase do estudo. Na terceira fase, 53 indivíduos com surdez de condução unilateral foram recrutados num departamento de

otorrinolaringologia dum hospital terciário. O TOC foi realizado para determinar a lateralização com diapasões de 128 Hz, 256 Hz, 512 Hz, 1024 Hz e 2048 Hz e com a oclusão total do ouvido não afetado. A audiometria tonal foi então realizada, separadamente e cega em relação aos resultados do TOC, para determinar o gap aéreo-ósseo (ABG) e a o limiar auditivo médio (PTA). Por fim, as respostas do TOC foram comparadas com o ABG e o PTA para determinar a sua precisão na quantificação do grau da perda auditiva.

Resultados: Na primeira fase do estudo, a oclusão do canal auditivo externo determinou uma elevação nos limiares auditivos com o aumento da frequência, desde 19.94 dB (250 Hz) até 39.25 dB (2000 Hz). A diferença dos limiares entre as condições de oclusão e não oclusão foi estatisticamente significativa, tendo aumentado de 10.69 dB (250 Hz) a 32.12 dB (2000 Hz). Não se verificaram diferenças estatisticamente significativas em relação ao género, ou entre examinadores. Na segunda fase do estudo, verificou-se uma elevação dos limiares auditivos com o aumento da frequência, de 20.85 dB (250 Hz, grupo 20–30 anos) a 48 dB (2000 Hz, grupo 60–70 anos). A diferença nos limiares auditivos entre as condições de oclusão e de não-oclusão foi estatisticamente significativa em todas as frequências; e aumentou de forma diretamente proporcional com a frequência, de 11.1 dB (250 Hz, grupo 20–30 anos) a 32 dB (2000 Hz, grupo 20–30 anos). Foram encontradas diferenças estatisticamente significativas para os três grupos etários, em todos os parâmetros, exceto na diferença a 500 Hz e na diferença total média. A perda auditiva média resultante da oclusão aos 500 Hz foi de 19 dB. Não se encontraram diferenças estatisticamente significativas entre o ouvido direito e o esquerdo, e entre géneros. Na terceira fase do estudo o TOC mostrou uma forte associação entre surdez e lateralização utilizando o diapasão de 512 Hz ($p = .001$). A sensibilidade do diapasão de 512 Hz na deteção de um PTA igual ou maior do que 35.6 dB foi de 94.6% e a especificidade foi de

75% para um valor preditivo positivo de 89.7% e um valor preditivo negativo de 85.7%, assumindo uma prevalência pré-teste de 69.8%.

Conclusão: O método de oclusão do canal auditivo externo utilizado demonstrou reprodutibilidade entre examinadores e com a idade. A oclusão elevou os limiares auditivos, sendo este efeito mais evidente nas frequências mais elevadas. O TOC permitiu predizer o grau de hipoacusia de condução unilateral. Se ocorrer lateralização para a ouvido afetado, é quase certa a evidência de hipoacusia de condução moderada ou grave. No TOC podem utilizar-se diapasões de 256 ou 512 Hz para o diagnóstico de hipoacusia ligeira, e o diapasão de 2018 Hz para a hipoacusia moderada. Contudo, no estudo do efeito da idade e com a validação do teste foi possível concluir que a utilização do diapasão de 512 Hz é a mais adequada para o TOC. A utilização deste teste pode permitir aos clínicos, em ambiente de consulta e de forma rápida, a distinção entre uma hipoacusia de condução de grau ligeiro e uma hipoacusia de condução de grau moderado ou superior.

Palavras-chave: Perda Auditiva Condutiva; audiometria; canal auditivo externo; oclusão; testes auditivos; testes imediatos; limiar auditivo

INDEX

INDEX OF TABLES

INDEX OF FIGURES

LIST OF ABBREVIATIONS

EAC	external auditory canal
COT	contralateral occlusion test
T	transmission
SN	sensorineural
R	right
L	left
RE	right ear
LE	left ear
-	negative
+	positive
TM	tympanic membrane
ME	middle ear
dB	decibel
Hz	hertz
TFT	tuning fork test
PTA	pure-tone average
ABG	air-bone gap
CSOM	chronic suppurative otitis media
ISO	international standard organization

1 INTRODUCTION

1.1 Organization of thesis

This thesis investigates the effect of the ear canal occlusion on pure tone hearing thresholds and the clinical applicability in validation of the contralateral occlusion test (COT). The thesis is presented as a series of papers published in international journals and book chapters but organized with the typical structure of a research document. The thesis consists of five chapters, each with a different research focus.

Thesis references are structured according to the style of the American Psychological Association (APA 6th, 2015) guidelines, since NOVA Medical School does not officially adopt any style or standard for bibliographic references. The APA style follows an author-date citation style. An author-data style was chosen because it allows for immediate identification of the referenced work. NOVA Medical School allows material from published journal papers to be included in the doctoral thesis. The inclusion of material from different publications and book chapters in this thesis may contain similar descriptions of concepts, test procedures and findings. For instance, the working principles of COT had to be clearly delineated for each publication. The anatomical terminology of the thesis is written according to the terminology style of the International Federation of Association of Anatomists and the Federative Committee on Anatomical Terminology (Terminologia Anatómica Internacional, 2001).

The development of a bedside testing suitable for use in a clinical setting, employing efficient and accurate measurement procedures, may contribute to the evaluation of the auditory system function. On the basis of this information, the clinician may decide which exams are most suitable and which strategies would provide the best results for improving diagnosis and treatment.

1.2 Bedside testing

Bedside testing, otherwise referred to as near-patient or point-of-care testing, is not new and remains an integral part of clinical practice (Price, 2001; Verghese, Charlton, Cotter, & Kugler, 2011). Many of the early diagnostic tests are initially performed at the bedside; this practice may accelerate clinical evaluation, reduce costs, and improve decision making (St John & Price, 2013). The key objective of bedside testing is to generate a quick result, so that appropriate treatment can be implemented soon, leading to an improved clinical and economic outcome (Ehrmeyer & Laessig, 2007; Porter, 2010; St John & Price, 2013).

Bedside or point-of-care tests are simple medical diagnostic tests that can be performed at the bedside. Its complexity has changed with the technology development. It may include clinical tests, laboratory testing or even portable radiologic tests. It includes testing at the bedside, outpatient sites, within or outside the hospital or clinics, or at home.

In otorhinolaryngological outpatients' units, hearing loss is one of the most common complains (Andrade, Albuquerque, Matos, Godofredo, & Penido Nde, 2013). Tuning fork testing allows a quick, qualitative assessment of hearing and also allows for the distinction between conductive and sensorineural hearing loss (Chole & Cook, 1988; Doyle, Anderson, & Pijl, 1984; Ruckenstein, 1995). The evaluation of patients with unilateral hearing loss can be quickly evaluated with Weber, Rinne and other tuning fork tests (TFTs). However, none of these tests really permits a quantitative hearing assessment. In this thesis it is described the development of a specific test for conductive hearing loss that allows some quantification of the hearing loss. The value and strength

of the study is based on the utility of a new quick test for physicians to screen hearing loss in their patients.

1.3 Medical diagnostic testing

A medical diagnostic test is defined as any approach used to collect clinical information, with the purpose of making a clinical decision (Leeflang, 2014). Bedside tests may use less elaborate instruments, such as tuning fork tests (TFTs), neurological hammers, medical questionnaires, and physical exam results; or they may use more elaborate instruments, such as portable electronic devices to control blood glucose, glycosuria, and temperature. Nowadays a myriad of portable electronic devices are available. A screening test evaluates if an apparently healthy individual may have a disease. A diagnostic test confirms the presence or absence of disease in an individual with signs and symptoms of a disease. In order to establish a definitive diagnosis, a medical diagnostic test is performed after a positive screening test.

The clinical decision produced by a medical diagnostic test is based on a statistical context:

1. The purpose of a test is to move the estimated probability of disease to one end of the probability scale (present/absent, pass/fail);
2. The clinical decision-making process is based on probability;
3. The probability is based on fundamental statistical concepts (sensitivity, specificity and accuracy) (STAT 509, 2019).

In a scenario of a simple test, there are two possible outcomes: a positive test result that would indicate the presence of disease, and a negative test result that would indicate the absence of disease (STAT 509, 2019). In this scenario of a simple test with two possible outcomes (positive and negative), the use of the test in a group of individuals (some with the disease and some without the disease) can produce four groups of results (Table 1). The 2 x 2 contingency table reflects the relationship between the results of the

test and the reference standard, at a given diagnostic threshold, i.e., a cut-off at which results are classified as positive or negative (David C. Miller & David, 2006).

Table 1- Possible outcome of the application of a test in a group of subjects (with and without the disease) in a 2 × 2 table.

	Disease	No Disease
Test Positive	TP - true positives individuals with the disease, and for whom the test is positive	FP - false positives individuals without the disease, but for whom the test is positive
Test Negative	FN - false negatives individuals with the disease, but for whom the test is negative	TN - true negatives individuals without the disease, and for whom the test is negative
	TP + FN = total number of individuals with the disease	FP + TN = total number of individuals without disease

However, in practice, all diagnostic tests are potentially fallible (Leeflang, 2014). Therefore, we use a comparative reference test, which is considered the gold standard. It is the method used to obtain a definitive diagnosis for a specific disease: it may be audiometry, biopsy, surgery, or other acknowledged standards (David C. Miller & David, 2006). It is used to compare with the other test in question and to evaluate its accuracy. In the present study, the diagnostic accuracy of the TFT for assessing hearing loss is measured by considering pure tone audiometry as the gold standard.

In a hospital environment, all individuals may appear quite similar; we do not know who has the disease and who does not. This is the reason why diagnostic tests are used, allowing clinicians to estimate the probability of disease based on the outcome of one or more diagnostic tests. There are useful measures for evaluating test validity of a diagnostic test. These measures were developed for evaluating the performance of a diagnostic test relative to the gold standard:

- Sensitivity is the proportion of individuals with the disease who have a positive test result, and it is estimated from the sample as TP/(TP+FN);
- Specificity is the proportion of individuals without the disease who have a negative test result, and it is estimated from the sample as TN/(FP+TN);
- Accuracy is the probability of the test to give correct results (positive in patients and negative in non-patients), and it is estimated from the sample as (TP+TN)/(TP+FP+FN+TN);
- Positive predictive value (PV+) is the probability of individuals with a positive test result of having the disease, and it is estimated from the sample as TP/(TP+FP);
- Negative predictive value (PV-) is the probability of individuals with a negative test result that are actually disease-free, and it is estimated from the sample as TN/(TN+FN);
- Prevalence is the probability of having the disease and is also referred to as prior probability of having the disease. It is estimated from the sample as (TP+FN)/(TP+FP+FN+TN) (STAT 509, 2019).

Sensitivity is described as "positive in disease" or "sensitive to disease." A test with high sensitivity empirically excludes the possibility of an individual having a specific disease, and it also means that there is a low proportion of false-negatives. Specificity is described as "negative in health" or "specific to health." A test with high specificity empirically confirms the possibility of an individual having a specific disease, and it also means there is a low proportion of false-positives. A positive result for a very specific test would give strong evidence in favor of diagnosing the disease of interest (Simundic, 2009).

Calculating sensitivity and specificity depends on the results of dichotomous tests. However, many tests are measured on a continuous numeric scale, and it is necessary to establish a cut-off value for positive and negative results in order to calculate the sensitivity and specificity. The positivity criterion is the cut-off value on a numerical scale that separates normal values from abnormal values. It determines which test results are considered positive (indicative of disease) and negative (disease-free) (STAT 509, 2019). Because the distributions of test values for diseased and non-diseased individuals are likely to overlap, there will be false-positive and false-negative results. When defining a positivity criterion, it is important to consider which mistake is worse (STAT 509, 2019).

Now let's suppose that a low value is selected as the cut-off point (Figure 1). It will cause the test to have a high sensitivity because empirically all individuals with the disease will have a positive test result. However, many of the healthy individuals also will have a positive result (false positives), so this cut-off value will generate a poor specificity.

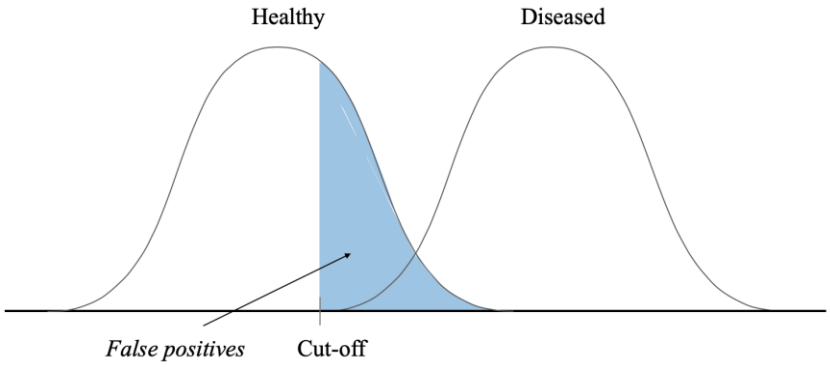

Figure 1- Test with a low cut-off value.

On the contrary, let's suppose that a high value is selected as the cut-off point (Figure 2). It will cause the test to have a low sensitivity because many of the diseased

individuals will have a negative result (false negatives). However, almost all of the healthy individuals will have a negative result, so the selected cut-off value will generate a high specificity.

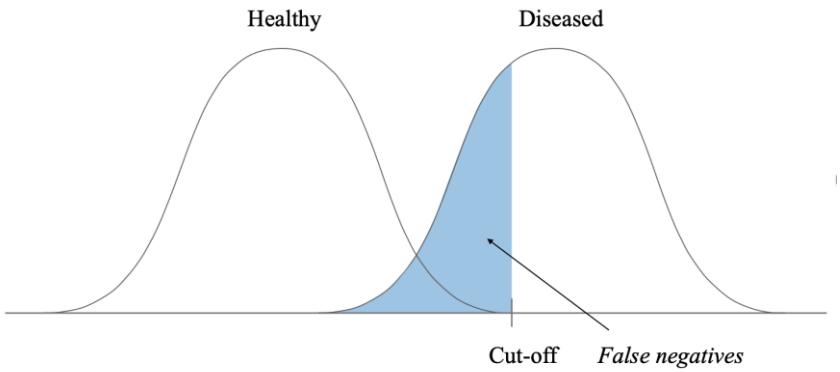

Figure 2- Test with a high cut-off value.

A receiver operating characteristic (ROC) curve reflects the operational characteristics of a diagnostic test with a continuous numeric variable (Figure 3). It is a graphical representation of the relationship between sensitivity and specificity. It is constructed by plotting the false positive rate (1-specificity) in the abscissa against the true positive rate (sensitivity) in the ordinate, for several options of the positivity criterion (Centre for Reviews and Dissemination, 2009). The true positive rate is the proportion of observations that were correctly predicted to be positive from the universe of all positive observations (TP/(TP+FN)). Likewise, the false positive rate is the proportion of observations that are incorrectly predicted to be positive from the universe of all negative observations (FP/(TN+FP) or 1-specificity). For example, in medical testing, when TN results are more desirable, set the positivity criterion to a point closer right on the ROC curve (increase specificity). On the contrary, if TP results are more desirable, set the positivity criterion to a point closer left on the ROC curve (increase sensitivity).

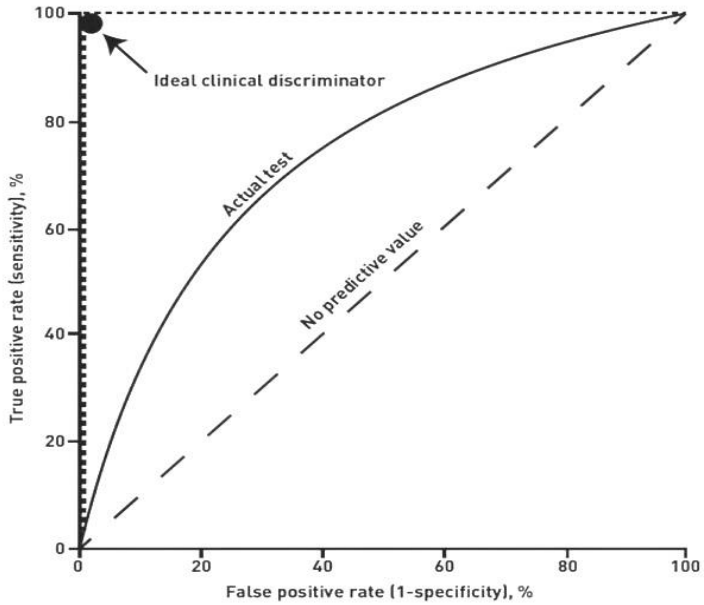

Figure 3- The ROC curve shows the relation between sensitivity (or TPR) and specificity (1 – FPR). (TPR: true positive rate, FPR: false positive rate).

Establishing a diagnosis is not a linear procedure. Ideally, medical diagnostic tests should provide correct results and should be non-invasive, without causing side effects. The use of simpler tests than the gold standard is done knowing that these results have some risk of incorrect diagnosis. This risk should be minimal and justified by the safety and convenience of simpler tests.

1.4 Hearing

Since earliest times, hearing has played an important role in the development of the human species. Knowledge and customs were transmitted by the power of the word from generation to generation. It was this sense of listening and communicating that differentiated the human species from others.

Hearing is the key to learning spoken language and it is important for cognitive development (WHO, 2016a). The ear is the human organ responsible for the sensory experience of hearing. Its function is to act like a link between the external environment and the nervous system, converting mechanical vibrations into an encoded nervous signal. The ear works like a biological microphone. In the microphone the vibration is converted into an electrical impulse, while in the ear the vibration is converted into a nervous impulse, which, in turn, is then conducted by the auditory pathways of the central nervous system (Alberti, 2001).

To perform the complex assignment of hearing, the auditory system works using three distinct functional steps (Figure 4). First, the acoustic stimulus is transmitted to the receptors as pressure changes. Second, the acoustic excitation is transduced into an electrical stimulus. In the third step, the electrical stimuli are processed to particularize the characteristics of the sound (frequency, intensity, and location) (Islam, Kok, & U., 2016). All of these functional steps have a topographic correspondence: the conductive structures (external and middle ear) transfers the acoustic stimulus from the environment to the inner ear and the sensorineural structures (cochlea, cochlear nerve, cochlear nucleus, medial geniculate nucleus, and auditory cortex) transduces the stimulus into a sensory response with activation of the associated nerve cells and central auditory nervous

system structures that finally decodes the auditory neural information (Islam, Kok, & U., 2016).

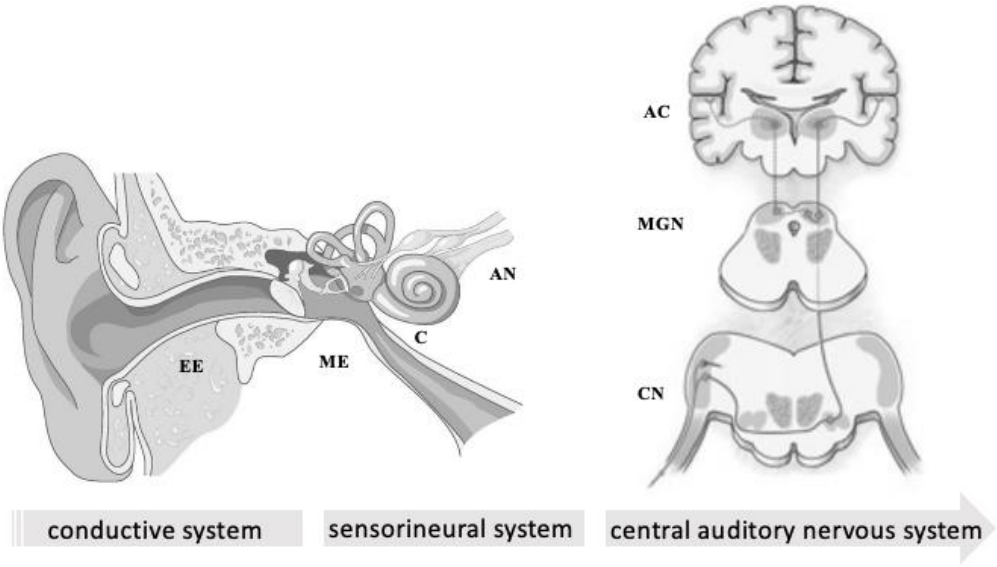

Figure 4- Diagram representing the three segments of the auditory system. The conductive system includes the external and middle ear (EE and ME). The sensorineural system includes the cochlea and the auditory nerve (C and AN). The central auditory nervous system includes the cochlear nucleus (CN, in brainstem), the medial geniculate nucleus (MGN, in thalamus) and the auditory cortex (AC).

1.4.1 Sound

Sound is a constant in our lives and is omnipresent, even in apparent silence (Raghu, 2018). Sound, from a physical point of view, is acoustic pressure produced by a vibrating body and the transmission of these vibrations (waves) to the surrounding medium (solid, liquid, or gaseous). The vibration of particles produces longitudinal waves of sound, arising from the source of sound and transmitting itself to that medium. Thus, we have waves of acoustic pressure produced by vibrating particles that are transmitted to the surrounding particles, and successively continuing from the sound source until the pressure dissipates. . Sound is also a kind of energy which causes an auditory sensation.

Sound can be the desired signal (e.g., speech), but often it is an unwanted noise (e.g., traffic). Contextually, noise is any undesirable and eventually harmful sound in the environment around us. Physically, noise is represented as a wave with random amplitude variations at each moment (Yost, 2000). Relevant in daily life, the signal-to-noise ratio (SNR) is established by the ratio between the significant information and the unwanted sounds and is generally expressed in decibels (dB). Additionally, we can consider two types of sound: simple sound and complex sound. Simple sound represents a single frequency (for example, the sound of a tuning fork), while complex sound incorporates multiple frequencies with varying intensity over time.

Sound waves are generated by any vibrating object and by the transmission of this vibration to the surrounding medium (solid, liquid, or gas). Particle activity results from this vibration, creating back and forth movements, with their transmission to the medium as a longitudinal sound wave (Maths and Physics, 2018). For each fixed distance from the sound source, the amplitude of this oscillation represents the sound pressure at that location. The sound pressure at a fixed distance from the sound source is calculated comparatively to the reference pressure, which is empirically the smallest pressure range that human ear can recognize. This relevant measure is denominated the sound pressure level (SPL), expressed in decibels, and is estimated as represented in the formula:

$$L_{p} = 20\ log_{10}\left(\frac{p}{p_0}\right)$$

where p is the sound pressure and p_0 is the reference pressure of 20 μPa. The wavelength is a consequence of the periodic characteristic of the sound wave and is calculated, for example, by the distance between two maximum pressure peaks in consecutive waves. Frequency is defined by the number of those periods that occur in one second. The relation between frequency and wavelength is estimated as represented in the formula:

$$f = \frac{c}{\lambda}$$

where *f* is frequency, λ is wavelength and *c* is the speed of sound. The speed of sound in air is 344 m/s at a temperature of 20◦C (Rossing, Moore, & Wheeler, 2002).

A vibrating tuning fork has the ability to generate a longitudinal wave (Miller, 1979). As the tines begin to vibrate, moving back and forth, these vibrations push on the surrounding air particles. When the tines move forward, they push air molecules linearly to the front creating an area of high pressure. Inversely, when the tines move backward, they create an area of low pressure. These forward and backward particle vibrations are transmitted longitudinally and mechanically to the surrounding air, generating areas in the air where the particles are compressed together and other areas where the air particles are spread apart (Maths and Physics, 2018). These described areas are called compressions and rarefactions, respectively. Due to these series of compressions and rarefactions in the air, between the fork and the listener, a sound wave propagates longitudinally through the medium. However, does a tuning fork also produce transversal waves? It probably produces both transversal and longitudinal waves. When the two tines are vibrating toward each other, they produce waves that collide in the middle of the "U" shape of the fork and form standing waves that travel down through the handle.

Sound has objective and subjective characteristics. Sound vibrations are objectively measured in terms of frequency, amplitude, and wavelength (Figure 5); additionally, sound is subjectively measured in terms of pitch, loudness, and timbre (Maths and Physics, 2018):

- Frequency is the number of vibrations (waves) per unit of time, also known as cycles per second. The unit of frequency measurement is the Hertz (Hz).

- Wavelength is the length of a single wave (vibration) and corresponds to the distance between equivalent points of two consecutive waves. For example, it can be measured by the distance from the peak of one wave to the peak of the following wave.

- Amplitude is the size of the vibration, and this determines how loud the sound is. Larger vibrations create a louder sound (intensity).

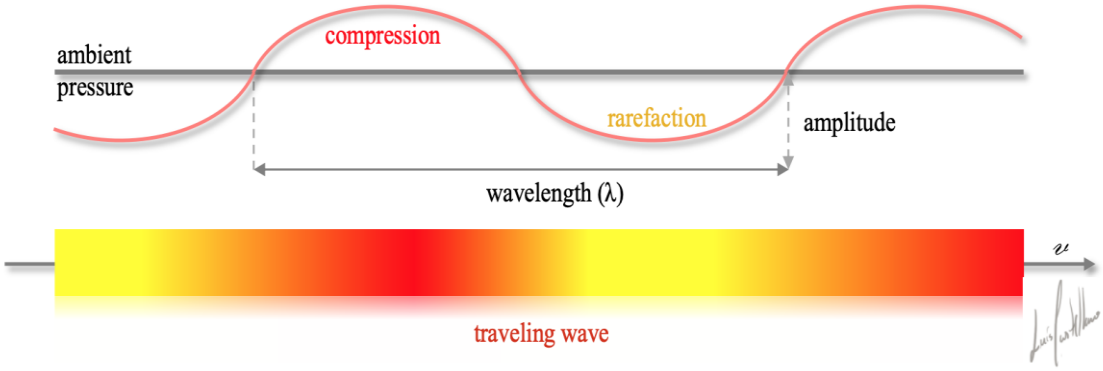

Figure 5- Representation of the propagation of a longitudinal sound wave in the air (v: propagation velocity).

- Pitch is the subjective perception if a particular sound has a higher or lower frequency. It is usually used in music to describe whether a note has a high or low pitch. Frequency is an objective scientific unit of measurement, while pitch takes into account the subjective perception of the frequency in relation to other frequencies.

- Loudness is the subjective perception whether a particular sound has a higher or lower volume. Intensity is an objective scientific unit of measurement, while loudness takes into account the subjective perception of the intensity within the context of other sounds.

- Timbre defines the "quality of a sound" or its "color". This feature allows different instruments have different sounds. Even though a clarinet and a cello can play the same tone with the same volume, they sound differently, and we can easily differentiate them. Every note that is played by the instrument is actually a smooth mixture of harmonics, and they are blended together so well that we do not hear them as separate. Instead, the harmonics give the note its color.

1.4.2 Anatomy of the ear

The ear consists of three different parts: the external, middle, and inner ear. The external ear includes the auricle and the external auditory meatus or external auditory canal (EAC), and its function is to collect and transmit the sound to the tympanic membrane (TM) (Alberti, 2001). The visible portion of the external ear is also named pinna, which in Latin means “feather” (Browning, Swan, & Chew, 1989). A relevant part of the auricle is the concha, which is perfectly designed to receive incoming sound waves and to funnel them into the external acoustic pore and EAC (Weber, Deschler, & Sokol, 2006). Although the EAC is not completely visible to the eye, it is nevertheless a component of the external ear.

An average human EAC is 26 mm long and 7 mm in diameter. The lateral portion of the EAC constitutes one-third to one-half of the total EAC length and is formed by a soft cartilaginous structure lined with skin with 0.5 to 1.0 mm of thickness. The skin has ceruminous glands and hair follicles (Moller, 2006). The ceruminous glands produce cerumen, which maintain the ear canal clean and protect it the from insects, bacteria and fungi. The inner portion of the EAC has a bony structure with thin (0.2 mm thick) skin tightly attached to the bone. This skin is easily injured or ruptured (Alvord & Farmer,

1997). The physical barrier between the external auditory canal and the middle ear is the TM.

The middle ear (ME) is an air-filled cavity that is medial to the TM and includes the Eustachian tube, the tympanic cavity (TC), and the mastoid cells. The ME cavity lies medial to the TM and is located within the temporal bone. The cavity of the ME can be compared to an imperfect box of 2 cm^3 (Figure 6). The TM forms the greater part of the lateral wall of the ME. The labyrinth is the most relevant structure that shapes the medial wall of the ME.

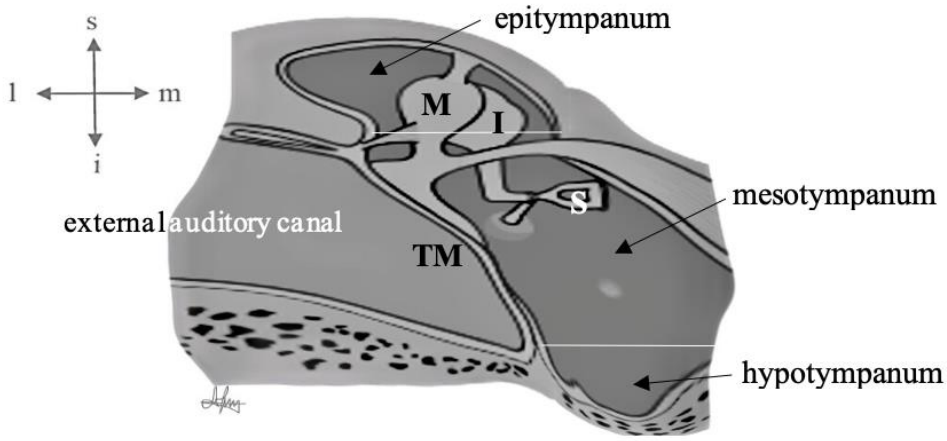

Figure 6- Regions of the tympanic cavity (epitympanum, mesotympanum and hypotympanum) with the tympanic membrane (TM) and the ossicles: malleus (M), incus (I), and stapes (S). The guidance axes are shown in grey (s: superior, i: inferior, l: lateral, m: medial) (right ear).

The anatomy of the medial wall is determined by the inner ear: the bulge of the promontory is the most identifiable landmark of the inner wall of the ME, formed by the basal turn of the cochlea; above the promontory, the oval window of the cochlea, covered by the stapes, is bordered superiorly by the Fallopian canal enclosing the facial nerve; under the promontory, slightly posterior, is the membranous round window and niche of the cochlea. The superior wall of the ME, called the tegmental roof, consists of a very

thin (<1 mm in some places), bony, irregular plate that limits the ME cavity from the cranial cavity (middle cranial fossa). The ME cavity is normally filled with air and communicates to the outside via the nasopharynx by Eustachian tube, that is located at the anterior wall of the ME. The ME encloses the ossicular chain, which transfer the vibrations of the TM to the oval window to be received by the cochlea (Seikel, 2009).

The TC is comprised of three regions: the hypotympanum, mesotympanum, and epitympanum. The hypotympanum is the region of the TC located below the inferior limit of the TM. The mesotympanum corresponds to the TC region medial to the TM. The epitympanum or attic is the region of the TC located above the superior limit of the pars tensa of the TM In its posterior part, the TC is connected to the mastoid through the *aditus ad antrum*.

The TM separates the external ear from the ME. The TM has a lateral inclination: therefore, its lower limit is more medial than its upper limit. Consequently, the TM draws an acute angle with the inferior wall of the CAE and an obtuse angle with the superior wall of the CAE, and the superior wall of the EAC is shorter than the inferior wall. The angle between the TM and the upper and posterior wall of the EAC is 140°, while the angle between the TM and the lower and anterior wall is 30° (Figure 7) (Paço, 2003; Teru et al., 2019). This angle is narrower in the newborn, when the TM is almost horizontal.

The TM is somewhat conically shaped and inwardly displaced by ~2 mm, with its external base and vertex pointing medially towards the ME. The vertical measure of the TM ranges from 8.5–10 mm, whereas its horizontal measure ranges from 8–9 mm. The complete area of the TM is 85 mm2, but functionally only 55 mm2 is relevant (Moeller, 2006). TM is a translucent and smooth membrane, with a medium thickness of 0.074 mm and a mass approximating 14 mg (Teru et al., 2019). It has a layered architecture with an

external epidermal layer (stratified squamous epithelium), an intermediate connective layer (lamina propria), and an inner epithelial layer (cuboidal respiratory mucosal epithelium). The external layer is continuous with the skin of the EAC. The respiratory mucosal layer is uninterrupted with the mucosa of the ME.

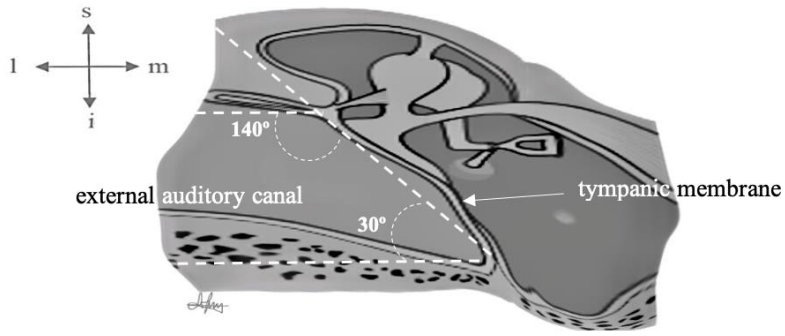

Figure 7- The tympanic membrane has a specific inclination, forming angles of 140º and 30º with the superior and inferior walls of the external auditory canal, respectively. The guidance axes are shown in grey (s: superior, i: inferior, l: lateral, m: medial) (right ear).

The TM is composed of the *pars tensa* and the *pars flaccida* (Paço, 2003). The *pars tensa* is the inferior larger portion of the TM and the *pars flaccida* is the smaller, superior portion of the TM. The lamina propria of the *pars tensa* has collagen fibers oriented in various directions. In the periphery of the *pars tensa* there is the annular ligament, a fibrous thickening of the TM firmly attached to the bony sulcus of the tympanic ring. The lamina propria of the *pars flaccida* has loose connective tissue and elastic fibers, which may explain its flaccid consistency (Teru et al., 2019).

There are several types of fibers with different orientations in the constitution of *pars tensa*: most are oriented radially and circularly; some are parabolic and a minority is oriented transversely (Figure 8) (Shimada & Lim, 1971). The concentration of fibers is higher in the center (next to the spatula). The radial fibers travel from the periphery towards the umbo and the *manubrium*. The circular fibers travel concentrically from one

edge to the other edge of the *manubrium*. The parabolic fibers travel from the lateral process of the malleus to the to the anterior or posterior quadrants, between the radial and circular fibers. The transversal are horizontal fibers located only in the inferior quadrant.

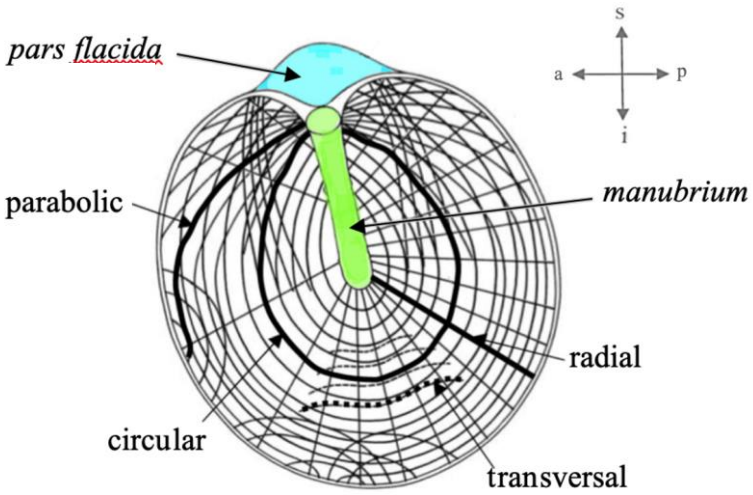

Figure 8- Fibers arrangement of the *pars tensa* in the TM. The guidance axes are shown in grey (s: superior, i: inferior, a: anterior, p: posterior) (left ear).

The acoustic energy received by the TM is conducted to the fluids of the inner ear by the three ossicles: malleus, incus and stapes, that function as a chain (Figure 9). The malleus is the largest ossicle, comprising five regions: *manubrium*, lateral process, anterior process, neck, and head. The incus is interposed between the malleus and the stapes. The incus comprises the body, a short limb and a long limb, with the lenticular process. The stapes comprises the head, the neck, two *crura* (limbs), and the footplate. The ossicular chain has two articulations between the ossicles (incudomalleolar and incudostapedial joints) and a third joint between the stapes footplate and the cochlea (stapedovestibular joint). Some Portuguese colleagues conceptualize the complex comprising the tympanic bone, malleus, and TM as a joint, calling it the "tympanicomalleal joint" (Gilberto, 2019).

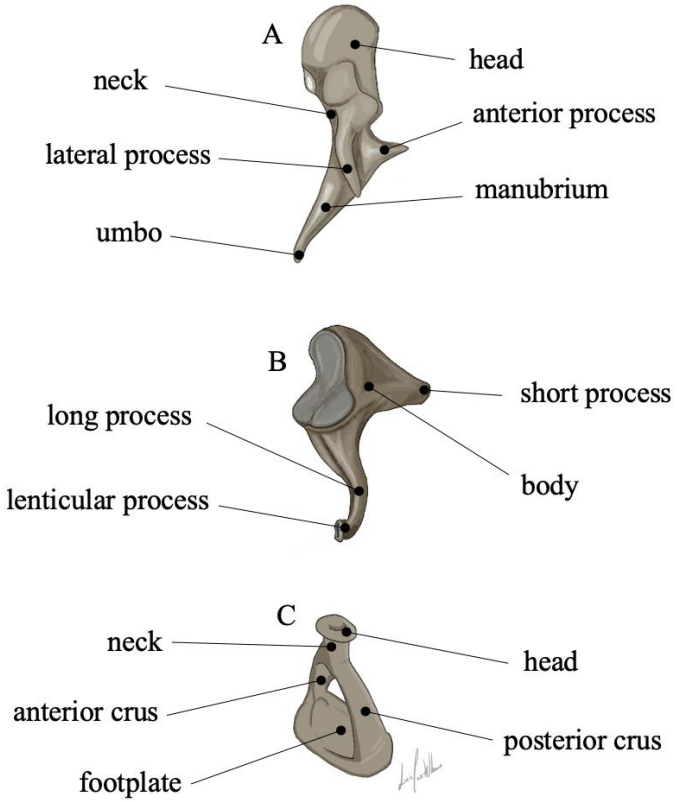

Figure 9- Anatomical representation of the three ossicles of the middle ear with malleus (A), incus (B) and stapes (C).

The ossicular chain is sustained by two muscles (tensor tympani muscle and stapedius muscle) and several ligaments (four malleus ligaments and two incudal ligaments), which hold the ossicular chain in position, allowing microscopic movements of the ossicles. The tendon of the tensor tympani muscle is attached to the *manubrium* of the malleus, displacing the *manubrium* medially when the muscle contracts. Most of this muscle is within a bony canal above the osseous portion of the pharyngotympanic tube.

The stapedius muscle is the smallest of all skeletal muscles. Its tendon is attached to the head or the neck of the stapes, limiting the displacement of the ossicle when the ossicular chain is vibrating, protecting the cochlea from the excessive acoustic stimulation. The malleus is hold by superior, anterior, posterior, and lateral ligaments, and the incus by posterior and inferior ligaments.

The pharyngotympanic tube (i.e., Eustachian tube) is a canal that communicates the nasopharynx with the tympanic cavity, equalizing the pressure between the tympanic cavity and the outside. In addition, during opening, mucus is transported through the Eustachian tube to the nasopharynx. An adult Eustachian tube is approximately 35 mm long. The tympanic extremity of the tube, which comprises approximately one-third of the tube, is made of bone, whereas the pharyngeal extremity is composed of cartilage (Moller, 2006).

The inner ear (labyrinth) includes the bony and membranous labyrinth. The bony labyrinth is enclosed within the petrous region of the temporal bone, and is formed by the cochlea, the vestibule and the three semicircular canals (superior, lateral, and posterior). The membranous labyrinth is a fragile and extremely complex structure located within the bony labyrinth, formed by the cochlear duct (inside the cochlea); utricle and saccule (inside the vestibule) and the membranous semicircular ducts (inside the semicircular canals). The membranous labyrinth is surrounded by perilymph and filled with endolymph, which is produced by the *stria vascular*i of the cochlea (Moller, 2006).

The cochlea is a spiral structure that is coiled two-and-three-quarter's rotation around its axis, the modiolus. Its functional spiral shape is space-saving, emphasizes sound resonance, and, if elongated, would reach a length no longer than 30 mm. The base of the cochlea arises from the *vestibulum*, the apical portion is the cochlear cupula or apex, and in the modiolus lies the cochlear nerve. Three parallel chambers reside within the cochlea, namely, the *scala vestibuli* (ascending spiral), the *scala tympani* (descending spiral), and the *scala media* (cochlear duct) (Figure 10). The vestibular membrane (Reissner's membrane) divides *the scala vestibuli* from the cochlear duct. The basilar membrane, in turn, divides the cochlear duct from the *scala tympani*. The *scala vestibuli*

communicates with the *scala tympani* at the level of the helicotrema, located at the apex of the cochlea, and the *scala tympani* ends at the round window, which serves as an escapement for the sound wave (Don, Kwong, & Katz, 2002).

The cochlea is filled with liquid whose total volume is merely 0.2 mL. The *scala vestibuli* and the *scala tympani* are filled with a fluid rich in sodium (Na^+) called perilymph, which is similar to cephaloraquidian fluid. The cochlear duct is filled with a fluid rich in potassium (K^+) called endolymph, which resembles intracellular fluid. The organ of Corti is located inside the cochlear duct and contains the receptor cells of the auditory system, the so-called hair cells (Monteiro & Trigueiros, 2018).

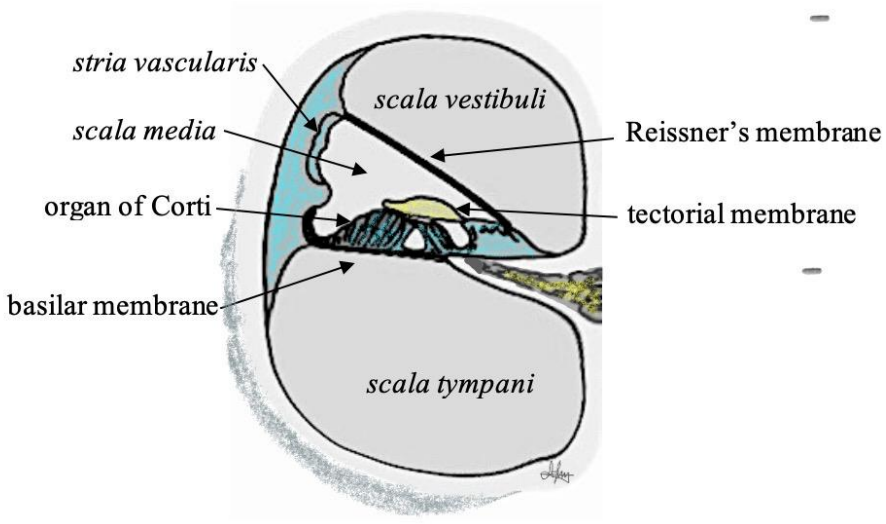

Figure 10- Schematic diagram of the cochlea with the three chambers: the *scala vestibuli* (ascending spiral), the *scala tympani* (descending spiral), and the *scala media* (cochlear duct).

The hair cells located inside the organ of Corti are responsible for the sensory elements of hearing, transducing mechanical sound vibrations into neural impulses. There are two types of hair cells: inner and the outer hair cells, according to their position in the organ of Corti in relation to the modiolus. The total number of inner hair cells in the the organ of Corti has been estimated at 3,500. They are organized in a single row and have

a piriform shape with a central nucleus (Monteiro & Trigueiros, 2018). The inner hair cells possess a more homogeneous morphology and size than do the outer hair cells.

The total number of outer hair cells is estimated to be triple the number of inner hair cells (~12,000), and they are organized in three rows and possess a cylindrical shape with a basal nucleus (Moller, 2006). At the top of the hair cells there are sensory bundles —the so-called stereocilia — that project upwards to the tectorial membrane, a gelatinous membrane located above the stereocilia in the *scala media*. Stereocilia are distributed with a gradual increase in height, becoming longer inside, on the edge of the modiolus. These cilia are interconnected (by apical and lateral links); thus, they all move together against the tectorial membrane. Stereocilia are in strong connection with the tectorial membrane. This membrane is responsible for the deflection and hyperflexion of the stereocilia during the vibration of the basilar membrane. Stereocilia show a different morphology at the top of the hair cells; they are V-shaped in inner hair cells and W-shaped in outer hair cells. Below the stereocilia, the cuticular plate serves as the foundation of the stereocilia at the hair cells (Monteiro & Trigueiros, 2018). Into the organ of Corti, there are also several types of supporting cells (Deiters, Hensen and Claudius cells), which have a role in the cycle of K+ and glutamate.

The cochlear nerve starts at the spiral ganglion neurons located in the organ of Corti, where the nerve branches make synaptic contact with the hair cells. Therefore, the cochlear nerve originates in the cochlea and extends to the brainstem, where its fibers synapse at the level of the cochlear nuclei. The cochlear nerve contains approximately 30,000 fibers. There are two types of fibers: the type I fibers (90–95%) are mostly afferent, with each inner hair cell being innervated by 10–15 of these fibers, while the

type II fibers (5–10%) are efferent, with each outer hair cell being innervated by 10 of these fibers (Monteiro & Trigueiros, 2018).

The central auditory pathways structures follow the auditory nerve, peripherally originated. These structures are the cochlear nuclei, the superior olivary complex, the lateral lemniscus, the inferior colliculus, the middle geniculate body, and ultimately terminating in the auditory cortex. The auditory nerve has afferent fibers (myelinated type I neurons), which transmit sound information originating mostly from the inner hair cells centrally, and efferent fibers (non-myelinated type II neurons), which relay information coming from the brain to the outer hair cells (Malmierca & Hackett, 2010).

1.4.3 Physiology of the ear

Hearing is the process by which the ear captures sound vibrations from the environment and transforms them into nerve impulses transmitted to the auditory cortex, in which sounds are interpreted. Sound waves are generated by vibrating objects; sound waves are transmission of their vibrations into the surrounding air. The auditory system can link the physical characteristics of sound with subjective aspects, such as pitch and loudness.

Pitch is a subjective perception of sound frequency and is measured in cycles per second or Hertz (Hz). Normal hearing subjects have a sensorial frequency spectrum ranging from approximately 16 to 20,000 Hz; however, the ear is more sensitive to frequencies emitted by human speech (1,000–4,000 Hz). Human infants may be able to hear frequencies above 20 kHz, but in adulthood the upper limit is reduced to 15 to 17 kHz (Rossing et al., 2002).

Loudness is a subjective perception of sound intensity that is connected to the pressure created by the sound waves on the TM. This pressure is measured in decibels (decibel sound pressure level [dB SPL which is a logarithmic unit of measurement used to express sound intensity relative to a given reference level. The reference level for dB SPL is 20 micropascals (20 μPa). Given the variability in human hearing capacity, the need for the creation of a dB unit suitable for use in clinical settings has arisen. Decibel as a measure of hearing level (dB HL) is commonly used in audiology, and it refers to the dB level on the audiometer. The range of human hearing extends from 0 to approximately 120 dB HL, which is the level at which sound is perceived as painful (Rossing et al., 2002). Figure 11 shows the relationship of dB SPL with dB HL in normal-hearing listeners. The minimum audible pressure amplitude at the threshold of hearing is ~10^{-5} Pa. The pressure increment associated with the threshold of pain is >10 Pa, which is one million times the pressure of the hearing threshold (Roeser & Clark, 2007).

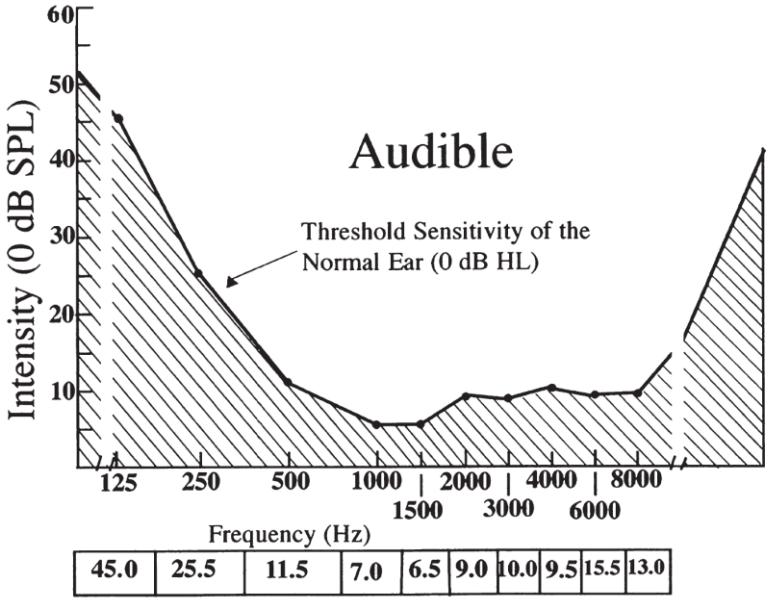

Figure 11- Average thresholds across frequency in decibel sound pressure level (dB SPL), corresponding to 0 dB HL. The solid black line represents the average auditory threshold in dB SPL at each audiometric frequency. For example, at 125 Hz, 0 dB HL = 45 dB SPL, and at 1000 Hz, 0 dB HL = 7 dB SPL. (Adapted from Roeser, RJ, and Clark, JL. Pure-tone tests. In Audiology: Diagnosis. 2nd ed. New York: Thieme Medical Publishers, Inc.2007)

The energy of the sound undergoes four transformative steps in order to stimulate the central nervous system (Figure 12). First, the ME, which includes the TM and ossicles, acts as an impedance adapter, transferring the acoustic pressure from air to liquid (inner ear). Second, the acoustic pressure is transformed into waves of fluid inside the cochlea. Third, the travelling wave formed in the basilar membrane stimulate hair cells tonotopically. Finally, hair cells transduce sound into cochlear nerve impulses, which are transmitted to the primary auditory cortex, the brain's ultimate hearing center. Sound is only consciously perceived when nerve impulses reach the auditory cortex (Kaas, Hackett, & Tramo, 1999).

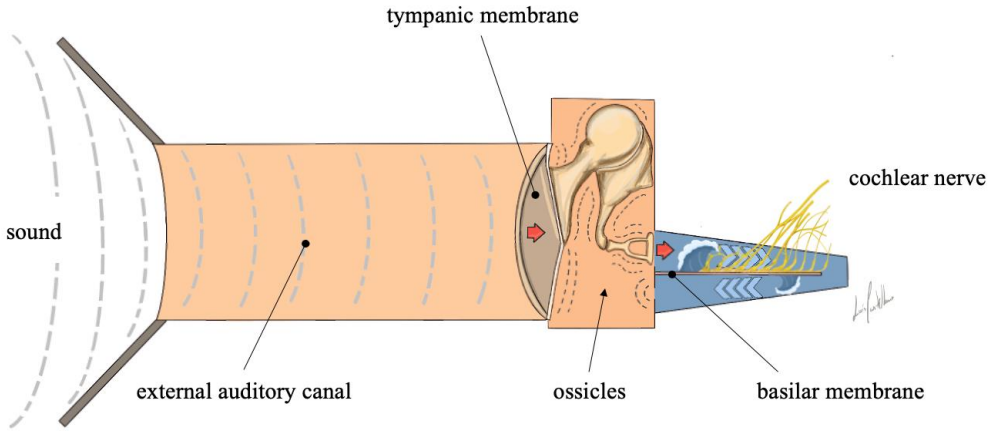

Figure 12- Diagram of human peripheral hearing following the path that sound energy takes through several transforming steps to stimulate the central nervous system.

The auricle, due to its anatomical configuration that is provided by the cartilage, functions as a sound receiver. The auricle behaves differently in response to frequencies lower or higher than 1 kHz. In the first case, sounds are not influenced by the auricle; however, for frequencies higher than 1 kHz, particularly for speech frequencies of 2 to 4 kHz, the sounds are considerably amplified. This behavior can be compared to a funnel, reflecting and directing the sound energy to the EAC (Everest, 2001). These direct and reflected signals arrive at the EAC mainly in phase. This synergistic effect starts at frequencies above 1 kHz. For higher frequencies, there is a small delay in the entry of the

reflected waves into the EAC, which causes phase cancellation or its destruction. The greatest interference occurs at higher frequencies, which produces an “auricle notch”, located around 7 to 10 kHz (Everest, 2001).

Anatomically, the EAC can be thought of as a tube that is opened at one end (at the concha) and closed at the other end (at the TM). The EAC acts as a resonator, causing sound energy at certain frequencies to vibrate with greater amplitude relative to amplitudes at other frequencies. The specific resonant frequency at which the sound energy is boosted is determined by the length of the tube in which the greater the length of the EAC, the lower its resonant frequency. The resonance frequency is consistent with the resonance characteristics of a tube closed at one end. The EAC of an adult increases the vibratory amplitude (sound wave energy) at frequencies of ~3,300 Hz (Hixon, 2018). As with all resonators, this energy boost reaches not only the peak frequency but also to the surrounding frequencies.

The ossicles play a critical role in the auditory system. Sound is conducted from the TM to the inner ear by the three ossicles, which act as a lever that increases the pressure on the TM at the oval window by as much as 30 times (Erminy, Skanavi, Van Den Abbeele, Avan, & Bonfils, 1995). This amplifying effect is attributed mainly to the difference in areas between the TM and oval window, while the ossicle’s lever effect provides a force-multiplying factor of ~1.5 (Rossing et al., 2002). The sound wave produces a vibration in the tympanic–ossicular complex, which functions as a whole until the base of the stapes is reached. As a result of the lever mechanism, the footplate performs an inward–outward movement in the oval window of the cochlea; consequently, the vibration of the entire ossicular chain and of the footplate generates pressure waves in the fluid-filled cochlear cavity.

The inward and outward stapes movement at the oval window produces fluid waves inside the cochlea (Figure 13). These waves are transmitted longitudinally through the *scala vestibuli*, then from the helicotrema to the *scala tympani*, and lastly, they dissipate at the round window. Waves are conducted transversally through the Reissner's membrane into the fluid of the cochlear duct at which they stimulate the basilar membrane, creating a wave that travels from the base to the apex (traveling wave). The basilar membrane resonates sequentially from high frequencies at the base of the cochlea to low frequencies at the top of the cochlea (tonotopy) because of its physical characteristics: the width of the basilar membrane increases, and the tension decreases from the base to the apex.

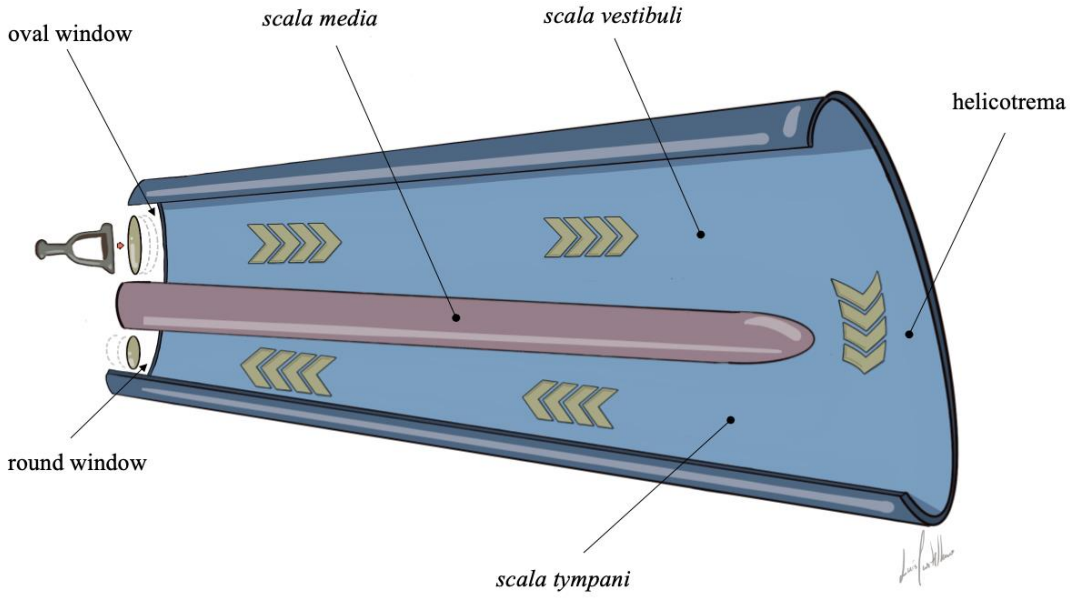

Figure 13- The sound vibrations cause fluid waves inside the cochlea, and a traveling wave is transmitted longitudinally through the three chambers until it dissipates in the round window. The yellow arrows represent the movement of the perilymph.

The movement of the basilar membrane is transmitted to the hair cells of the organ of Corti. This upward movement pushes the inner hair cells against the tectorial membrane, which causes folding of their stereocilia. Consequently, there is an opening

of the ion channels in the inner hair cells. These channels act as transducers and convert mechanical displacement into action potentials. The propagation of action potentials in hair cells releases a neurotransmitter to the neural fibers of the auditory nerve. Thus, an action potential is generated and transmitted by the fibers of the auditory nerve to the cochlear nuclei and up until the auditory cortex, where the stimuli are decoded as recognizable sounds.(Daniels et al., 1996; Kaas et al., 1999).

The outer hair cells have a central core without a cytoskeleton, which gives them strength, flexibility, and electromotility in response to stimuli, providing a refinement in frequency sensitivity. Outer hair cells are called the cochlea amplifiers because they tune the cochlea for a given frequency (Rossing, Moore, & Wheeler, 2001). The outer hair cells contract rhythmically in reaction to auditory stimuli. They respond to a specific frequency according to their location along the length of the basilar membrane. The central efferent response produces contraction of all of the outer hair cells of the basilar membrane, except in the region where the inner hair cells are being stimulated.

After the frequency, the sound location is the second information treated by the human auditory system. Using both ears to listen provides information about intensity, direction and distance of sound sources. The binaural location is established on a comparison of the sound stimulus, as received differently in each of the ears. Which side is the sound more intense? Which side does the sound arrive first? These two main clues for sound location are the interaural time difference (ITD) and the interaural level difference (ILD) and this mechanism occurs when the auditory nerve fibers intersect in the brain stem.

An ITD delay occurs when the sound takes longer to reach the ear farthest from the source. An ILD occurs when part of the sound energy is attenuated by the head, to

reach the distal ear. The human auditory system is capable of interpreting ITD and ILD separately at different frequencies, but ILD is more prominent at higher frequencies. This is how the system detects the approximate location of the sound source (Blauert, 1997; Kaas et al., 1999; Rossing et al., 2002; Stern, Brown, Wang, Wang, & Brown, 2006). An effect known as the cocktail party effect also exists. Because of this effect, a normal-hearing individual can alternate their attention between different conversations at will in a crowded, noisy environment. This central process allows for automatic adjustments of the arrival time and sound intensity of different signal sources, so that the signal deemed most relevant passes into the cortex, and those that fail to meet these criteria of relevance are erased by feedback loops (Alberti, 2001).

An acoustic stimulus is transmitted to the auditory cortex via two pathways: the primary auditory pathway, transmitting auditory information from the cochlea, and the non-primary pathway (reticular sensory pathway) transmitting all types of sensory information (Monteiro & Trigueiros, 2018). The first is shorter (only 3–4 relays), faster (due to large myelinated fibers), and terminates in the primary auditory cortex. Each relay nucleus performs specific decoding and integrating tasks. In the second pathway, small fibers bridging the cochlear nuclei are connected with the reticular formation by small fibers and the auditory message is incorporated into other sensory messages. There are also pathways leading to wakefulness and motivation centers and to vegetative and hormonal systems. Conscious perception requires the integrity of both pathways (Monteiro & Trigueiros, 2018). The Central Auditory Processing network permits some of the characteristics of hearing: (1) sound interpretation, (2) hearing discrimination and discrimination in noisy spaces, (3) ability to block out unwanted sounds, and (4) spatial sound localization.

1.5 Hearing Loss

1.5.1 Epidemiology

Hearing loss is a significant source of morbidity worldwide. In adults, disabling hearing loss refers to a hearing loss > 40 dB in the best hearing ear, and in children, a hearing loss > 30 dB in the best hearing ear. The World Health Organization (WHO) estimated that in 2019 four hundred and sixty-six million people worldwide would have disabling hearing loss and of those, thirty-four million would be children (WHO, 2016b). Hearing loss is the sixth highest cause of disease based on the Disability Adjusted Life Years (DALYs) in high-income countries in 2001; however, hearing loss was not in the top ten causes in low- and middle-income countries because other issues, such as perinatal conditions (lower respiratory infections, ischemic heart disease, and human immunodeficiency virus/acquired immunodeficiency syndrome [HIV/AIDS]) were more significant (Lopez, Mathers, Ezzati, Jamison, & Murray, 2006). Moreover, hearing loss has a significant impact on the quality of live in people who suffer from it. Several studies carried out by WHO concerning the global burden of disease included hearing loss, in which it was found that hearing loss onset when reaching adulthood was the second leading cause of Years Lived with a Disability (YLDs) and represented 4.6% of total YLDs (Mathers, Smith, & Concha, 2000). Additionally, hearing loss onset when reaching adulthood was estimated to account for 1.7% of the total global burden of disease based on the DALY scale (WHO, 2004).

Hearing loss is the partial or total incapacity to hear sound in one or both ears. The audiometry is the tool used to determine the degree and type of hearing loss. This section presents hearing loss classification based on type, etiology, and degree.

1.5.2 Types of hearing loss

Hearing loss can be categorized into three groups: (1) conductive, (2) sensorineural, or (3) mixed (Figure 14).

Conductive hearing loss is produced by the damage to the sound conductive system, which may occur at any location from the pinna to the hair cells. The damage to the sound conductive system makes the signal reaching the cochlea weaker than it should be. That is, the cochlea has normal hearing capacity, but the acoustic signal reaching the cochlea is attenuated. Consequently, in conductive hearing loss, there is an increase in the air conduction threshold with normal bone conduction threshold. The degree by which the signal is attenuated by the damage is expressed by the magnitude of the air-bone-gap (Gelfand, 2009).

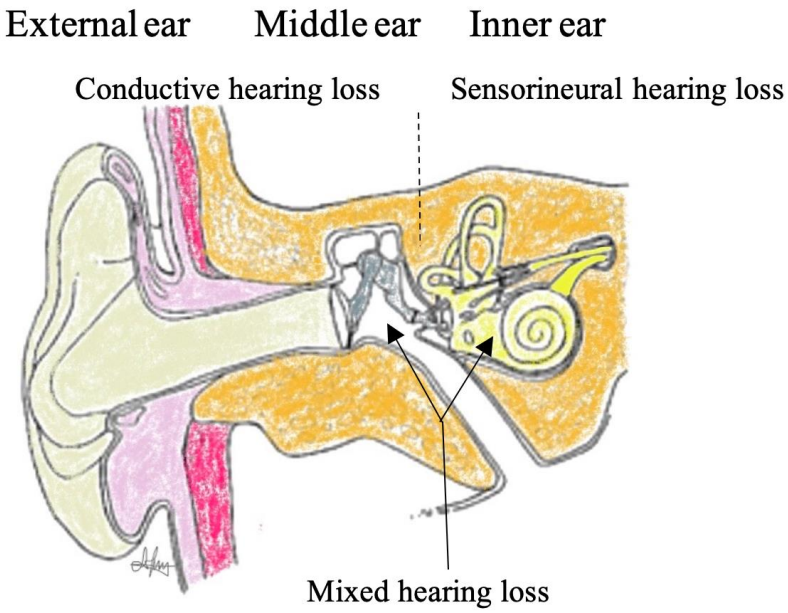

Figure 14- Anatomical correlation of the different types of hearing loss with the different affected regions of the ear.

Sensorineural hearing loss is produced by sensory or neural damage to the auditory pathway, which may occur at any location from the cochlea until the auditory cortex. Sensory or neural damage may occur either by lesions of the inner ear (cochlear)

or lesions of the auditory pathway (retro-cochlear). In sensorineural hearing loss, there is an increase in the bone conduction threshold that overlaps the air conduction threshold; this process usually represents an irreversible hearing loss (Gelfand, 2009).

Mixed hearing loss is the association of conductive with sensorineural hearing loss in the same ear. Mixed hearing loss may be produced by the presence of two separate disorders in the same ear (such as noise-induced hearing loss plus otitis media) or by a single disorder that concurrently affects the conductive and sensorineural systems (such as head trauma or otosclerosis) as describe by Gelfand, 2009. In mixed hearing loss, there is an increase in bone and air conduction thresholds with an air bone gap > 10 dB.

1.5.3 Etiology of hearing loss

Hearing loss can be classified according to its etiology. Conductive hearing loss is produced by disorders that affect the external and middle ears. Examples of external ear disorders are malformations (such as atresia of the EAC or microtia), otitis externa, exostoses, trauma, presence of a foreign body, tumors (such as squamous cell carcinoma and osteomas), and cerumen impaction. Examples of middle ear disorders producing conductive hearing loss are otitis media with effusion, otosclerosis, chronic otitis media, cholesteatoma, trauma of the temporal bone, middle ear ossicular malformations, and glomus tumors (Torres & Backous, 2010).

Sensorineural hearing loss is produced by disorders that affect the inner ear and the central auditory neural pathways. There are two categories of sensorineural hearing loss, genetics and non-genetic Among genetic causes, one-third are syndromic (hearing loss associated with anomalies in other organ systems) and two-thirds are non-syndromic (hearing loss is the only clinical abnormality). Among non-genetic causes, there are presbycusis, noise-induced hearing loss, ototoxicity, meningitis, Meniere's disease, barotrauma,

acoustic neuromas, meningiomas, autoimmune diseases, multiple sclerosis, and stroke (Arts, 2010).

1.5.4 Degree of hearing loss

The WHO and International Bureau for Audiophonology (BIAP) classifications are the most frequently used in clinical practice to describe the degree of hearing loss. The WHO Working Group on Prevention of Deafness and Hearing Impairment Planning defined the degree of hearing loss by classifying it into four distinct groups (Table 2). The frequencies of 500 Hz and 1, 2, and 4 kHz in the better hearing ear were used to calculate the degree of hearing loss because these frequencies are generally considered to be part of the speech frequency range. Mild hearing loss was defined as an average threshold level between 26 and 40 dB, moderate hearing loss defined as an average threshold level between 41 and 60 dB, severe hearing loss defined as an average threshold level between 61 and 80 dB, and profound hearing loss as an average threshold level $\geq$ 81 (WHO, 1991).

Table 2- Classification of hearing loss by degree (WHO, 2016).

Degree of Hearing Loss	Intensity of Sound (dB)
Normal	≤ 25
Mild	26-40
Moderate	41-60
Severe	61-80
Profound	≥ 81

In 1997, WHO defined disabling hearing impairment (DHI) separately for adults and children under the age of 15 considering the frequencies of 500 Hz and 1, 2, and 4

kHz in the better hearing ear. For adults, it includes moderate to profound hearing loss or an average threshold of $\geq$ 41 dB in the better hearing ear. For children under the age of 15, DHI was defined as an average threshold of $\geq$ 31 dB in the better hearing ear at the referred frequencies (WHO, 2002).

Another audiometric classification of hearing loss is the recommendation by the BIAP (Table 3). The average hearing loss was calculated after considering the loss in dB at several frequencies, including 500, 1000, 2000, and 4000 Hz. A frequency that was not perceived was considered as a loss of 120 dB. For each ear, the threshold was calculated by the arithmetic average of the results (in dB) at the four frequencies and rounded up to the nearest unit. In the case of an asymmetric hearing loss > 15 dB, the average level of loss (expressed as dB) was obtained by multiplying the result from the best ear by seven and by three from the worst ear, and the result of the sum of these two values is then divided by ten.

Table 3- Classification of hearing loss by degree (BIAP, 1996).

Degree of hearing loss		Intensity of Sound (dB)
Normal		$\leq$ 20
Mild		21-40
Moderate	1st degree	41-55
	2nd degree	56-70
Severe	1st degree	71-80
	2nd degree	81-90
Very severe	1st degree	91-100
	2nd degree	101-110
	3rd degree	111-119
Cofosis		$\geq$ 120

1.6 Diagnostics of hearing loss and hearing measurements

1.6.1 Otoscopy

Otoscopy is a procedure that allows visual inspection of the EAC and TM through an otoscope. This inspection is necessary to evaluate the condition of the TM, to identify changes behind the TM and to identify possible signs of infection or obstructions on the EAC (Don, Kwong, & Katz, 2002).

1.6.2 Tuning fork tests

Because their importance in this work, the TFTs are discussed in a separate section (see ahead, section 1.7).

1.6.3 Timpanometry

Tympanometry is a test that allows an objective evaluation of ME function. It tests the pressure of the ME and the tympanic-ossicular compliance, creating variations in the air pressure of the EAC. A tympanogram can detect ME problems such as TM perforations, changes in pressure in the TC, fluid in the TC, flaccidity of the TM or rigidity of the TM (due to the presence of scar tissue) or ossicles (due to otosclerosis, infections or malformations) (Don et al., 2002). The Jerger classification is used for tympanometry findings (Figure 15), which is the most widespread approach to describe clinically the tympanograms.

A type A tympanogram has a well-defined peak in compliance within +50 to -100 mm water (dPa) and a height compliance within 0.3 to 1.5 cm^3. To be considered normal, the position of the compliance peak on pressure and the height dimension must be within normal range. A type B tympanogram has a flat pattern with no peak in compliance, with little or no apparent change in its pattern in response to pressure variations in the EAC.

This type is generally associated with otitis media with effusion, due the presence of fluid within the ME, or can be found in ears with TM perforation but, in this case, there is an increase in EAC volume. A type C tympanogram has a peak in compliance within the negative pressure region beyond -100 mm water (dPa). This type is usually found among patients with Eustachian tube dysfunction. Variations of a type A tympanogram have a peak compliance within 0 to -100 mm water (dPa) but with variable height compliance. If the peak compliance is less than the lower normal limit of compliance (less than 0.3 cm3) is type As, and if the peak exceeds the superior normal limit of compliance (more than 1.5 cm3) is type Ad.

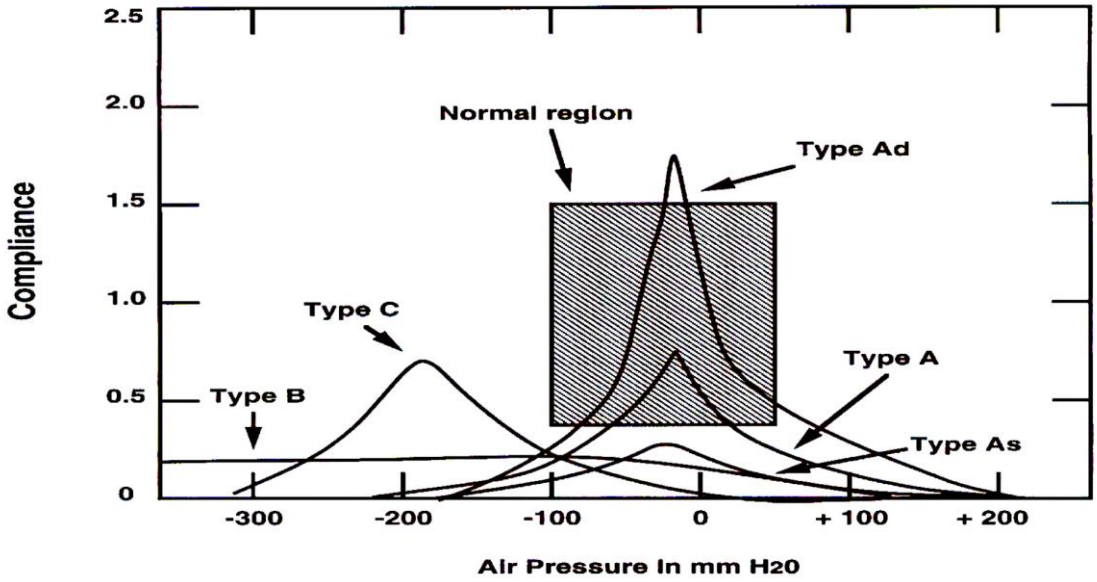

Figure 15- Classification of tympanograms according to Jerger. (Adapted from Jerger JF. Clinical experience with impedance audiometry. Arch Otolaryngol 1970;92:11-24)

1.6.4 Pure tone and speech audiometry

Pure tone audiometry is the gold standard hearing test for the clinical evaluation of hearing. It allows a subjective but reproducible evaluation of the hearing thresholds at different frequencies. The audiogram correlates the intensity (dB HL) and the frequency

(Hz) as a graph. The correlation of these two dimensions is the standardized way of representing the hearing thresholds. These thresholds start at the audiometric zero (0 dB HL) up to a maximum of 120 dB HL and can vary in the standard frequencies from 125 Hz to 8000 Hz. The hearing threshold is defined by the lowest intensity at which the examinee identifies the presence of the signal at a given frequency at least 50% of the time ((Don et al., 2002). The reference zero for the calibration of audiometric equipment (based on the average hearing thresholds of normal individuals) is standardized, with values defined in BS EN ISO 389-1:2000. The audiogram represents the gold standard test for determining the degree and type of a hearing loss. A hearing loss is defined by an increase in hearing thresholds and there are several classifications used in clinical practice to describe the degree of a hearing loss.

A standard pure tone audiometry allows to define two types of hearing thresholds:

- Air conduction thresholds, which are measured using sounds presented by phones or insert ear phones. The signal reaches the inner ear through air conduction, via the external and ME.
- Bone conduction thresholds, which are measured using sounds presented by a bone vibrator usually applied behind the ear on the mastoid bone. The signal reaches the inner ear through skull bone conduction, bypassing the external and ME.

Under normal conditions, the air and bone conduction thresholds overlap. The increase in air conduction thresholds can be caused by a problem in either or both the external/ME and the inner ear (Figure 16). However, the increase in bone conduction thresholds can only be caused to a problem in the inner ear or in the central auditory neural pathways (sensorineural loss). Impaired air conduction thresholds combined with

normal bone conduction thresholds indicate a conductive hearing loss. The ‘air-bone gap’ (ABG) is the difference between air and bone conduction thresholds.

Speech audiometry is complementary to pure tone audiometry and is indispensable. While pure tone audiometry only indicates the absolute auditory thresholds of pure tone sounds, speech audiometry determines speech intelligibility and discrimination. This type of information is particularly important in the candidacy for hearing aid and in the diagnosis of retrocochlear pathologies. For children up to 5 years behavior tests (visual reinforcement audiometry for the younger and play audiometry for the older children) allows to determine hearing threshold and quantify the hearing loss. Behavior audiometry replaces the user feedback button with an activity instead. For non-cooperative individuals hearing assessment by these methods cannot be achieved.

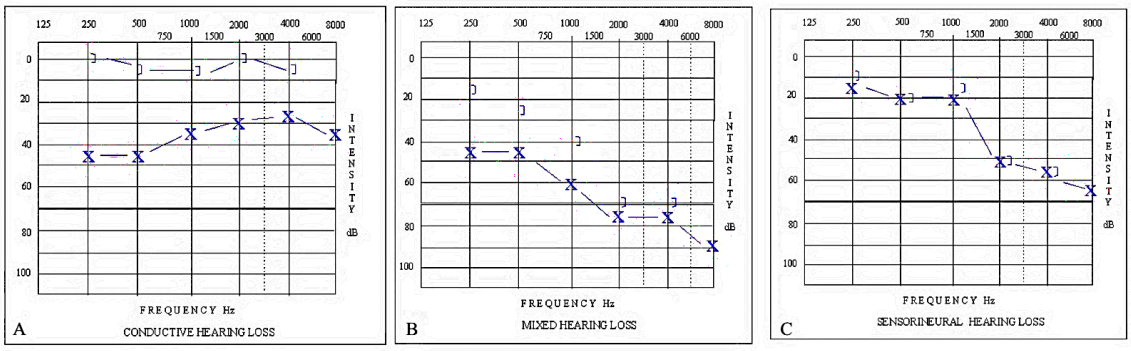

Figure 16- Audiograms depicting: A) mild rising conductive hearing loss, B) mixed sloping hearing loss, and C) high-frequency sloping sensorineural hearing loss. Left ear. (Adapted from https://emedicine.medscape.com)

1.6.5 Distortion Product Otoacoustic Emissions

Otoacoustic emissions (OAEs) are sounds produced by the cochlea, spontaneously or evoked, that can be recorded by a microphone adapted into the EAC. The test evaluates objectively, simply and non-invasively the cochlear function. The presence of OAEs are an indicator of normal cochlear function. OAEs are known to be

absent with inner ear damage (Kemp, 2002). They can be used in neonatal hearing screening and for any suspected cochlear damage.

Spontaneous OAEs are non-audible sounds produced at cochlea without stimulus in about 40% of individuals, by contraction of the outer hair cells. Inversely, evoked OAEs are produced in response to a stimulus. If the stimulus is two tones close in frequency, they generate in the inner ear the Distortion Product Otoacoustic Emissions (DP OAEs). If a stimulus is a click, it generates transient OAEs in the inner ear.

The DP OAEs are an indicator of the presence of auditory active processes in the cochlea (Don et al., 2002). The absence of DPOAEs is usually attributed to outer hair cells damage. No DPOAEs are expected to be recorded, regardless of the underlying pathology, if the hearing loss is greater than 50 dB SPL (Gorga et al., 1997).

1.7 Tuning fork tests

TFTs, when combined and interpreted, may be a reliable initial diagnostic tool. They should be used for situations before or when audiometry is not available and should always be interpreted in the light of their sensitivity and specificity. Its importance continues vital as bedside testing by shortening decision time and improving decision making.

TFTs are performed with a tuning fork - in Portuguese *diapasão*, from the Latin *diapason*, which means octave (interval between the seven notes on the musical scale) (Figure 17). The tuning fork was invented by John Shore, in 1711 for musical purposes (Bickerton & Barr, 1987). In medicine it is used in several specialties. Until the appearance of the clinical audiometer, the tuning fork testing was the only way for hearing evaluation of the patients.

Figure 17- Tuning forks used in the medical practice, with different frequencies (128, 256, 512, 1024, 2048, and 4096 Hz).

The advantages of tuning fork are to produce a pure tone, vibrate really close to its fundamental frequency and produce very small overtones. The term overtone is used

to characterize all resonant frequencies above the fundamental frequency, that are part of a sound produced by a vibrant object. This term 'overtones' does not contain the fundamental frequency. A large number of overtones may be produced along with the fundamental tone. Harmonics are also frequencies greater than the fundamental frequency of a sound, but they are integer multiples frequencies of the fundamental frequency. It can be concluded that a harmonic may be an overtone, while an overtone may or may not be a harmonic.

1.7.1 History

The knowledge that human ear can identify sounds either by air or bone conduction was established right from 17th century onwards, but clinical utility of this knowledge only started with the invention of the tuning fork, attributed to the musician John Shore in 1711. Using the fork, clinical otologists began to believe that they had a diagnostic tool for qualitative assessment of hearing loss by differentiating the source of the disease in middle ear or in the inner ear. The German physicist Ernst Florens Friedrich Chladini (Wittenburg,1800) analyzed the vibrating characteristic of tuning forks and being named as the father of experimental acoustics (Tschiassny, 1946).

TFTs emerged in the nineteenth century with the physiologist Ernest Heinrich Weber (1825) and his brother Eduard Friedrich Weber as clinical examination tool tests. They performed psychophysical studies and verified the lateralization of tuning fork, in people with unilateral hearing loss. Jean Pierre Bonafont (1835) applied the Weber experiments in the clinical evaluation of patients with hearing loss. Subsequently, Adolf Rïnne (1855) compared the air conduction with the bone conduction. Lucae and Bezold used the terms positive and negative to differentiate the results of the Rinne test. Dagobarth Schwaback (1855) reported the prolongation of bone sensations in cases of

conductive hearing loss. Subsequently, Albert Bing (1891) and Marie Ernst Gellé developed other tests with tuning forks (Tschiassny, 1946).

1.7.2 Principles

As a bedside test, TFTs are performed in daily clinical practice in order to obtain a quick, qualitative and subjective assessment of an individual's hearing. These tests can be performed by using tuning forks of several frequencies (128, 256, 512, 1024, 2048 and 4096 Hz) (Johnson, 1970).

The tuning fork consists of a sonorous stem (bifida, with two parallel tines), a base and sometimes a footplate. The frequency of the sound emitted by a tuning fork depends on its geometrical elements: the frequency is inversely proportional to the length of the branches and directly proportional to its thickness. Therefore, the narrower and longer are the branches of the tuning fork, the lower its frequency of vibration (low frequency sound). Inversely, the thicker and shorter are the branches, the higher its vibration frequency (high frequency sound). The tuning fork vibration produces fundamental sound harmonics, which quickly become extinct, so that the sound becomes a simple frequency (pure tone). 128 and 256 Hz tuning forks could be provided with clamps; when used in testing bone conduction, the clamps may allow damping the over-tones of the instrument and long-lasting vibration.

The technical characteristics of a tuning fork should ideally include the construction of a good metal alloy, vibrate at a specified fundamental frequency, maintain vibration for as long as possible and produce the least number of overtones. Aluminum tuning forks are more commonly used in the clinic; however, the steel forks possibly may detect a smaller ABG (MacKechnie et al., 2013; White, 1974; Yuksel & Kemaloglu, 2017). Tuning forks with frequencies below 256 Hz show great vibration and are felt

more than heard and, therefore, are not used in the clinic. TFTs using forks with frequencies above 1024 Hz show rather poor sensitivity and hence are not clinically used. The frequency of the sound emitted by the tuning fork is fixed, not suffering from the "de-calibration" problem of the audiometers. The sound intensity emitted by the tuning fork depends on the intensity of the stimulation (percussion).

The TFTs allow to distinguish between sensorineural and conductive hearing loss. Its importance remains essential as bedside testing and allow the confirmation of audiometric results. Bedside tests are performed immediately, allow diagnoses, and consequently reduce decision time, costs, and improve decision making. Bedside tests obtain a prompt result, in order to diagnose and treat immediately, with good results and minimal costs (Johnson, 1970).

1.7.3 Procedures

All tests should be performed in a quiet room and in cerumen-free ears. The tuning forks should be vibrated against a soft structure (such as the elbow or rubber pad) or between the fingers (thumb and forefinger). If beaten against a solid object, additional non-harmonic frequencies can be produced (Samuel & Eitelberg, 1989; Stevens & Pfannenstiel, 2015), distorting the pure tone generated by the tuning fork, and the result is an unreliable test. The fork should be struck at the intersection of upper third and lower two thirds, because it is the part of the fork where maximum vibration is obtained (Samuel & Eitelberg, 1989).

The 256 and 512 Hz tuning forks should be used because they are the ones with the most reliable results (Browning & Swan, 1988; Chole & Cook, 1988; Kelly, Li, & Adams, 2018). Low frequency tuning forks, such as 128 Hz, produce greater bone

vibration; those of high frequency, lose vibratory energy rapidly and are more difficult to use in ears with moderate or severe sensorineural loss.

Before performing the tests, it is important to explain the procedure of the tests and understand the clinical setting of patients' complaints. The tests most frequently used in the clinic are the Rinne, Weber and Bing tests (Table 4). Once popular, Schwabach and Gellé tests are no longer in clinical practice use.

Table 4- Results of the Weber and Rinne tests.

Weber test	R	L	R	L	I	I	R/L	R/L
Rinne test R	-	+	+	+	-	+	+	-
Rinne test L	+	-	+	+	-	+	+	-
Hearing loss	T in RE	T in LE	SN in LE	SN in RE	N or T bilateral	N or SN bilateral	SN bilateral (with better RE/LE)	T bilateral (with better RE/LE)

R: right; L: left; I: indifferent; RE: right ear; LE: left ear; -: negative; +: positive.
N: normal; T: transmission; SN: sensorineural.

1.7.4 Rinne test

The Rinne test is a TFT that compares air conduction and bone conduction. Air conduction is better than bone conduction in a healthy ear. The test is performed by firmly applying the base of a vibrating tuning fork to the patient's mastoid. When the patient signals that the sound of the fork has stopped, the fork is quickly transferred close to the EAC. The test is considered positive if the sound is still audible when the fork is transferred (air conduction is better than bone conduction) and negative if the sound is no longer audible (bone conduction is better than air conduction). In the particular situation of profound unilateral hearing loss, the sound may be heard by the normal contralateral

ear through bone conduction, leading to a false positive Rinne situation. Alternative methods of the Rinne test can be performed, but these methods are not as reliable as the original. An alternative method of the Rinne test is performed by reversing the order of tuning fork placement (first on the canal, then on the mastoid). Another alternative method is performed by placing sequentially the vibrating tuning fork on the mastoid and the EAC, and then simply asking where it is better audible (bone conduction versus air conduction).

The vibrating tuning fork should be placed parallel to the EAC axis (not perpendicular), because this produces a greater amplitude of sound at the level of the TM (Butskiy, Ng, Hodgson, & Nunez, 2016).

Ideally the 512 Hz tuning fork is used, but the 256 Hz tuning fork may allow greater sensitivity and specificity in the results (Browning & Swan, 1988). The specificity of the test is high when the conductive hearing loss is greater than 30 dB (90%), but decreases as the ABG reduces. The false positive rate is 20% in people with normal hearing (Kelly et al., 2018).

1.7.5 Weber test

The Weber test is a useful TFT to patients who have unilateral or asymmetrical hearing loss. The test is performed by firmly applying the base of a vibrating tuning fork to the patient's midline (vertex region, frontal region, base of the nose or in the incisors with mandibular occlusion). The patient must be previously instructed to indicate whether the sound lateralizes to one of the ears or if it does not lateralize to any of the ears. Sound waves are transmitted equally to both cochleae through the skull. A unilateral sensorineural hearing loss makes the sound lateralize to the ear with the best cochlear function, while a unilateral conductive hearing loss makes the sound lateralize to the side

with the hearing loss. The results are reported depending on the lateralization of the sound: Weber on the right, on the left or indifferent (if there is no lateralization).

This test is very sensitive to detect asymmetrical hearing loss. However, the Weber test yields incorrect responses in 33% of the patients for accurately distinguishing a sensorineural from a conductive hearing loss (Bagai, Thavendiranathan, & Detsky, 2006; Stankiewicz & Mowry, 1979).

1.7.6 Bing test

The Bing test is a TFT used to differentiate conductive from sensorineural hearing loss. The test is performed similarly to the Weber test, but with EAC occlusion. This occlusion increases the sound of the tuning fork in the ear if there is a conductive hearing loss. The test is performed by placing a vibrating tuning fork in the center of the subject's forehead, with the EAC completely occluded. A normal individual or an individual with sensorineural hearing loss hears louder when the EAC is occluded and hears softer when the canal is not occluded (Bing Positive). An individual with conductive hearing loss will not realize any changes (Bing Negative).

The Bing test has a low sensitivity and specificity, and a low reliability of correctly identify a conductive hearing loss (57-66%) (Wilson & Woods, 1975).

1.7.7 Schwabach test

The Schwabach test is a TFT that can be used to differentiate between conductive and sensorineural hearing loss. The test is performed based on a comparison of the duration of the auditory sensation produced by the tuning fork. The tuning fork is applied to the patient's mastoid until the patient can't hear it anymore. This sound duration is then compared with the duration that the fork produced in a normal ear (examiner). There is a

normal Schwabach if both the subject and the examiner stop hearing the tone at approximately the same time. If there is a sensorineural hearing loss, the subject will hear the sound for a shorter period (decreased Schwabach). If there is conductive hearing loss, the subject will hear the sound longer than normal (extended Schwabach).

1.7.8 Gellé Test

The Gellé test evaluates the bone conduction by placing a vibrating tuning fork on the mastoid and increasing the air pressure in the EAC. EAC occlusion increases the middle ear and intralabyrinthine pressure and consequently decreases the mobility of the basilar membrane. This will empirically decrease hearing, but no changes will be expected in hearing if ossicles are fixed. Usually the test is performed with a Politzer balloon. In individuals with normal hearing, when the pressure in the EAC is increased by the Politzer balloon, a loudness decrease is verified for both air-conduction and bone-conduction (positive Gellé). In the presence of stapedial ankylosis, a loudness decrease occurs for air-conduction but not for bone-conduction (negative Gellé). The marked variability of the results and the inaccuracy of the test, despite several improvements, did not allow its application in the diagnosis of pathology of the middle ear (Dankbaar, 1970).

1.8 Literature review

1.8.1 The use of tuning fork tests

The Weber and Rinne TFTs were invented in the XIX century, and they have become irreplaceable for the screening and diagnosis of patients with hearing loss, even after audiometry tests were introduced. Since their inclusion in the standard clinical assessment for patients with hearing loss, these tests have helped to validate audiometric results and assess hearing loss severity and often to confirm surgical indication. For example, in clinical practice, otolaryngologists are usually trained to use TFTs to confirm the surgical indication for patients with otosclerosis, advising surgery for those whose Rinne test indicates enough conductive hearing loss that they will benefit from stapes surgery (Kelly, Li, & Adams, 2018).

1.8.2 The value of tuning fork tests

The literature is not clear or conclusive about some TFT properties, such as their ability to detect unilateral sensorineural hearing loss (SNHL) or CHL, the magnitude of the air–bone gap (ABG) that is necessary to make the Rinne test negative, whether these results depend on the tuning fork material or frequency, the characteristics of the tested individuals, or the technique that is used to perform each specific test (Kelly, Li, & Adams, 2018).

1.8.3 Influence of the tuning fork material

Despite the historical data that are associated with the usefulness of TFTs, some researchers performed additional studies to identify parameters that could increase the reliability of TFTs. These studies identified, for example, measurable differences in the results when using forks that are made of different materials (MacKechnie et al., 2013;

White, 1974; Yuksel & Kemaloglu, 2017). Steel tuning forks can detect a smaller ABG when compared to aluminum tuning forks (MacKechnie et al., 2013), and conductive hearing loss is more likely to produce a negative Rinne test with a steel tuning fork than with an aluminum fork. Statistical analysis showed that the probability that the Rinne test is negative is up to 50% for an ABG of 19 dB for a stainless steel fork, but this percentage is shown only at 27 dB with an aluminum fork (MacKechnie et al., 2013). Because the Rinne test is used to evaluate candidates for stapes surgery, these results may have clinical implications in choosing the material for the tuning forks that are used in the stapes candidacy surgery. Additionally, over time, some of the used aluminum tuning forks were reported to lose their physical properties, which are important for clinical TFT tests (Yuksel & Kemaloglu, 2017). This phenomenon was related to metal fatigue, which is common in aluminum products, because of the cyclic load. Fundamental frequencies of the recorded sounds that are produced by 512-Hz tuning forks that were used presented a high variability, from 0.19% to 74.15%, from the assumed fundamental frequencies, but this rate was lower (1.49%) when a 1024-Hz fork was used. Furthermore, decay times of the 512-Hz tuning forks varied from 5.41 to 40.97 seconds.

1.8.4 Influence of striking the tuning fork

Recommendations about how to strike the tuning fork to minimize the overtones are as follows: the 128-Hz and 256-Hz tuning forks must be struck against a soft surface, while this may not be necessary with the 512-Hz and 1024-Hz forks (Samuel & Eitelberg, 1989). The forks that are the most sensitive in producing overtones are the 128-Hz and 256-Hz forks. By striking the 128-Hz fork on a hard surface (wooden desk), 18 overtones were recorded, whereas against the elbow, only six overtones were elicited. Striking the tuning fork on the femoral condyle (which is the same consistency as the elbow), as

recommended by the Committee of the Section of Otolaryngology (1933), should elicit the same number of overtones. If the tuning fork is struck slightly distal to the elbow, at a point that is covered by the flexor muscle, a pure tone is recorded because this point has a similar consistency to the duster (soft on hard material). Similar results were recorded with a 256-Hz tuning fork. For Samuel & Eitelberg (1989), no overtones were produced by 512 Hz and 1024 Hz forks, no matter what material was used for striking. Similar results were achieved for Stevens & Pfannenstiel (2015), but the 512-Hz fork also produced overtones. Although the fundamental frequency of the tuning fork was the dominant frequency that was recorded, additional non-harmonic frequencies were observed when the 256-Hz and 512-Hz tuning forks were struck against metal and wood surfaces (Stevens & Pfannenstiel, 2015). To keep overtones to a minimum, it was recommended that the fork should be struck at a point one-third of the distance from the free end (Samuel & Eitelberg, 1989).

1.8.5 Influence of the vibrating tuning fork's orientation

The orientation of a vibrating tuning fork's tines also seems to affect the amplitude of the sound signal that reaches the TM (Butskiy, Ng, Hodgson, & Nunez, 2016; Lin et al., 2014). When the tuning fork tines were placed parallel to the EAC, there was a higher sound amplitude at the level of the TM, as opposed to when the fork was placed perpendicular to the EAC (Butskiy, Ng, Hodgson, & Nunez, 2016). The sound intensity (sound pressure level) that was recorded on the TM using the 512-Hz tuning fork with the forks placed parallel to the EAC was higher by 2.5 dB for the fundamental frequency and by 4.94 dB and 3.70 dB for the two harmonic frequencies (not fundamental) (1 and 3.15 kHz, respectively) compared with using the same fork frequency with the tines placed perpendicular to the EAC. Using a 256-Hz tuning fork in parallel with the EAC,

as opposed to perpendicular, was louder by 0.83 dB for the fundamental frequency (256 Hz) and by 4.28 dB and 1.93 dB for the two harmonic frequencies (500 and 4 kHz), respectively.

1.8.6 Tuning fork tests for children

The use of TFTs for children is controversial (Capper, Slack, & Maw, 1987; Yung & Morris, 1981). Yung & Morris (1981) concluded that TFTs are a cheap and effective way of diagnosing conductive hearing loss that is associated with serous otitis media in children over 4 years of age, and they found that it was not difficult to obtain cooperation and accurate results. Contradictory results were obtained by Capper, Slack, & Maw (1987). Their results showed that the sensitivity and specificity of the Rinne and Weber tests were poor, making these tests of little use in screening and diagnosis of children with glue ear. The results with the Rinne test showed that 19 dB was the level of conductive hearing loss at which most children changed from a positive to a negative response. However, the large number of "incorrect" responses makes it difficult to use the Rinne test in younger children, but the reliability of the test increases in older children. With the Weber test results, the level of interaural hearing loss at which lateralization occurred was 4 dB; however, children often guess, and it was recommended to use the Weber test in cases where a major difference in conductive loss (more than 20 dB) may be present.

1.8.7 Masking and tuning fork tests

Most TFT studies evaluated the ABG rather than the pure-tone average (PTA). The need for masking in audiometry is recognized but the need for masking in tuning fork testing has not been established (Burkey, Lippy, Schuring, & Rizer, 1998; Miltenburg, 1994), which is probably because it seems to be unnecessary and very difficult to perform.

1.8.8 Occlusion of the external auditory canal in physiological and pathological conditions

Although EAC occlusion is a common situation in daily life, there are few studies on this subject. The effects of EAC occlusion are significant because of the modifications that it may cause to hearing through interference in the variability of bone and air conduction. It can occur in various physiological or pathological conditions, including the presence of cerumen, exostosis, or an occlusion that is caused by the hearing aid mold. The prevalence of excessive cerumen varies between 5% and 10% in children and adults. Prevalence of EAC exostosis is very high in individuals who participate in water sports. The degree of EAC exostosis varies and it occurs in over two-thirds of individuals who participate in these activities. It is estimated that 3.8 million Americans (14.2%) aged 50 years and older with hearing loss use hearing aids.

1.8.9 Studies on occlusion of the external auditory canal

Despite the frequency of EAC occlusion in daily life, there are only two studies about the effects of EAC occlusion on hearing thresholds (Table 5) (Chandler, 1964; Roeser, Lai, & Clark, 2005). In both studies, the occlusion was performed with inorganic materials (gel and earplug). The use of finger pressure is different from previously described methods. Partial occlusion affects mainly high frequencies. Total occlusion significantly affects the low frequencies (< 1000 Hz). Data from Chandler (1964) referred to only two patients. Roeser et al. (2005) found the same pattern of pure tone threshold variations according to the percentage of EAC occlusion, but the study was performed with only five normal-hearing adults.

Table 5- Hearing thresholds difference (dB) depending on the frequency, with total occlusion of the external auditory canal, in the studies of Chandler and Roeser.

	Frequency Hz						
	250	500	1000	2000	4000	8000	average
Chandler (1962)	29	30	43	42	39	59	40.3
Roeser (2005)	30	37	38	43	46	52	41
difference	-1	-7	5	-1	-7	7	-0.7

Roeser et al. (2005) showed that the mean thresholds of pure tones changed as a function of the percentage of the EAC occlusion. Partial occlusions (40% and 60%) mainly affect the acute frequencies, and thresholds below 1000 Hz are only significantly affected when total occlusion occurs. These data are consistent with Chandler (1964).

The two studies revealed an increase in the hearing threshold when the EAC was completely occluded. Theoretically, the increase in the hearing threshold resulting from EAC occlusion can empirically be added to pre-existing hearing loss. This effect might also occur with aging and with any hearing configuration. The pattern of hearing loss generated by the EAC occlusion that we hope to observe in this study, especially at higher frequencies, may be similar to presbycusis. It should, therefore, result in a difference in the deficit. This might be of considerable significance when an individual is being fitted for a hearing aid because any amount of hearing loss resulting from the acoustic prosthetic adaptation must be artificially replaced by the hearing aid amplifier.

1.9 Objectives

A bedside test that may predict the degree of hearing loss in cases of unilateral conductive hearing loss was designed. After the confirmation of unilateral conductive hearing loss using the Weber and Rinne tests, the novel Contralateral Occlusion Test (COT) will be performed with the total occlusion of the external auditory canal (EAC) of the contralateral ear (the non-affected ear). This will produce a hearing loss on the non-affected ear that can be higher, lower, or similar to the hearing loss of the affected ear. In this scenario, the sound of a vibrating tuning fork placed in the forehead at midline will lateralize to the ear with the greater hearing loss or will not lateralize to either ear.

To validate the test, it is necessary to quantify and standardize the effects of the EAC occlusion on hearing in order to decide which tuning fork frequency is more appropriate for use in the COT. Moreover, it is mandatory to evaluate the reproducibility of the occlusion method, as performed by several examiners, and also the reproducibility with frequency and aging. EAC occlusion in COT is a simple, inexpensive, reproducible, and non-invasive method.

This research aims to develop and validate a bedside test with tuning forks suited to quantify the hearing loss in individuals with unilateral conductive hearing loss. The objectives of the study are:

1. To determine the effects of complete occlusion of the EAC on hearing thresholds in normal-hearing young adults in order to apply it in the COT.
2. To evaluate the reproducibility of the occlusion method (between examiners) in normal-hearing young adults.

3. To determine the best frequency of the tuning fork to distinguish between mild and moderate conductive hearing loss, depending on the hearing loss induced by the occlusion effect.
4. To determine the effects of complete occlusion of the EAC on hearing thresholds at different ages and to evaluate the reproducibility of the method with aging, in order to apply it in the COT. We also aim to determine the best tuning fork frequency for different ages.
5. To determine the accuracy of the COT in quantifying the degree of hearing loss, in the presence of unilateral conductive hearing loss. The secondary outcome is to compare the investigational test with reference methods (audiometry).

2 METHODS

This study was conducted in three phases. In each phase the characteristics of the participants, procedures and statistical analysis were different. In the following subchapters, the ethical aspects and methodology for each phase will be discussed:

1. The first phase involved the determination of the effects of complete occlusion of the EAC on hearing thresholds in normal-hearing young adults. The reproducibility of the occlusion method (between examiners) was evaluated in order to apply it in the COT. Depending on the hearing loss produced by this effect, the best tuning fork frequency to distinguish between mild and moderate conductive hearing loss was also evaluated.
2. The second phase evaluated the reproducibility of EAC occlusion at different ages and frequencies. The most reliable tuning fork frequency according to age was also determined.
3. In the last phase, the test was validated for its clinical applicability.

2.1 Overview of technical audiology

2.1.1 Tuning forks

Five standard aluminum tuning forks with varying fundamental frequencies were used: 128 Hz, 256 Hz, 512 Hz, 1024 Hz, and 2018 Hz (Figure 18). They were made of high-quality aluminum and non-magnetic, non-corrosive, and light weighted with high accuracy. We used aluminum tuning forks that are more applied in a clinical context despite steel forks might detect a lower ABG (MacKechnie et al., 2013). They fulfilled the technical features for an ideal tuning fork: made of a good metallic alloy, vibrate at the specified frequency, capable of maintaining the vibration for a long period, and do not produce any overtones (Doyle et al., 1984). The examiner was competent in tuning fork tests. The testing was performed as described:

- All tuning fork tests (TFTs) were presented inside a soundproof room under strict and controlled conditions for the most accurate results.
- The examiner previously instructed the patient on each of the tests. Direct and explicit instructions prevented misinterpretation by the patient.
- The examiner beat one of the tines of the tuning fork on a rubber surface or on his own elbow, holding the instrument by its stem. The hit should be in a distance two thirds of the way base of the tine.
- The examiner sequentially performed the following TFTs: Weber test, Rinne test, and contralateral occlusion test (COT).
- When performing the COT, the patient was asked where the tone was heard, centrally or towards the left or right ear.

Considering the historical data of TFT, we considered the past data regarding the performance skills, which increases the reliability in TFTs. We followed the recommendations about how to strike the tuning fork to minimize overtones: all tuning

forks with different frequencies were struck against a soft surface (Samuel & Eitelberg, 1989; Stevens & Pfannenstiel, 2015). We also followed the recommendations about how the fork should be struck at a point one third of the distance from the free end to keep overtones to a minimum (Samuel & Eitelberg, 1989). Finally, the tuning fork tines were placed with an orientation parallel to the ear because that may affect the amplitude of the sound signal (Butskiy, Ng, Hodgson, & Nunez, 2016; Lin et al., 2014). The placement of the tuning fork tines in parallel to the EAC, as opposed to perpendicular, results in a higher sound amplitude at the level of the TM (Butskiy, Ng, Hodgson, & Nunez, 2016).

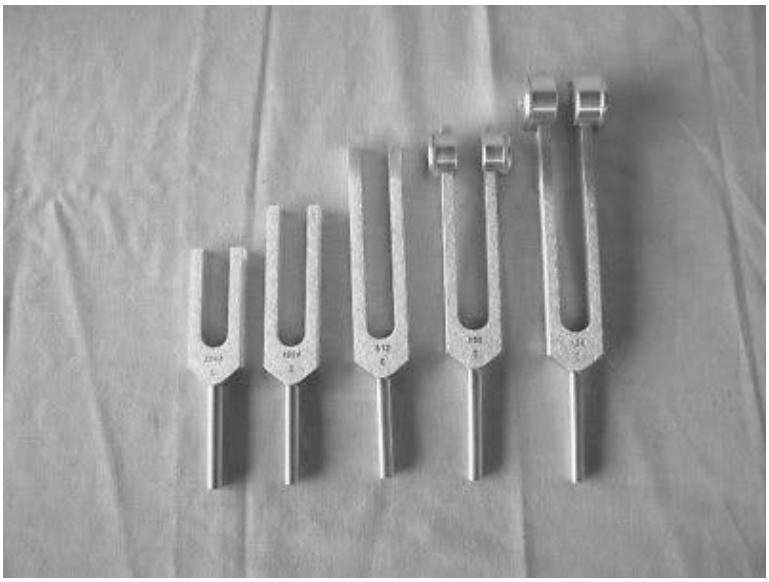

Figure 18- Tuning forks used in the study: 2048, 1024, 512, 256, and 128 Hz (left to right).

The tuning fork consists on a U-shaped sonorous stem (with two parallel tines), a base, and a footplate. Clinical tuning forks are tuned to the correct frequency at the factory, and the frequency in hertz is engraved on them. The frequency of the sound emitted by a tuning fork depends on its geometrical elements. The frequency is inversely proportional to the length of the tines and directly proportional to its thickness. The tuning

fork vibration produces fundamental sound harmonics, which quickly become extinct so that the sound becomes a simple frequency (pure tone).

Tuning forks with frequencies from 256 to 1024 were used most frequently: frequencies below 256 Hz are felt like a vibratory sensation, and frequencies above 1024 Hz are often not heard. The frequency of the sound emitted by the tuning fork is fixed and does not suffer from the "de-calibration" problem of the audiometers. The sound intensity emitted by the tuning fork depends of the intensity which it is stimulated (percussion). As mentioned before, there are also controversial differences in the results when using forks made of different materials (MacKechnie et al., 2013; White, 1974; Yuksel & Kemaloglu, 2017).

2.1.2 Pure-tone audiometry

Pure-tone audiometry is the gold standard test for obtaining hearing thresholds and define audiometric configuration. It can determine the degree and type of a hearing loss. We used pure-tone audiometry in our study to compare the audiometric results with the COT results. The measurements with occlusion of the external ear canal (EAC) were made in a free field inside the large calibrated soundproof room of the Department of Audiology of Egas Moniz Hospital.

The following equipment was used and certified according to the ISO 8253-3 : the Orbiter clinical audiometer (Madsen Electronics A/C; Herley, Copenhagen, Denmark), the 922 TDH39 earphones (Telephonics; Farmingdale, NY, USA), the ME70 noise-excluding headset (Madsen Electronics A/C; Herley, Copenhagen, Denmark), and the B-71 bone conductor (Radioear Corporation; New Eagle, PA, USA). Pure-tone measurements included air-conduction (AC) thresholds for octaves 250 to 8000 Hz and bone-conduction (BC) thresholds for octaves 500 to 4000 Hz (Appendix 7.6).

Pure tone audiometry uses pure tone sounds of different frequencies to quantify hearing, determining hearing thresholds at each frequency. The test procedures are defined by the standard ISO 8253-1 and follow an adaptive procedure (ISO, 2010). The test is performed in a quiet environment (soundproof room) and the examinee signals each time he hears a sound (i.e., presses a button). For each frequency, the sound is presented at different intensities, in order to determine the hearing threshold at that frequency. The test starts at the frequency of 1000 Hz, then proceeds up to 8000 Hz and, finally, the lowest frequencies are tested. If the patient has a hearing aid or tinnitus, warble tones should be used as the stimulus signal (Martin & Clark, 2014). A warble tone is a sound modulated at the frequency, which means that the frequency of the stimulus fluctuates around the tested frequency. Normative data for normal hearing have been established with large-scale testing in young people and are outlined in ISO 389 (ISO, 1998).

Pure-tone audiometry is performed successively in air conduction and in bone conduction. Air conduction measurements use headphones or insert earphones and evaluate the degree of hearing loss, but does not discriminate conductive, sensorineural, or mixed hearing loss. Bone conduction measurements stimulated the cochlea by bone vibrations, using a vibrator placed on the mastoid process. Bone conduction measurements determine the sensorineural hearing sensitivity. Because the conductive apparatus (EAC and ME) is bypassed, these structures do not influence the results of bone conduction measurements. The air-bone gap (ABG) is the difference of the hearing thresholds at air-conduction and at bone-conduction measurements. The ABG quantifies the conductive involvement of the hearing loss. For example, if the hearing loss is sensorineural, the ABG is null (zero) (Martin & Clark, 2014).

Pure-tone audiometry uses test frequencies in the range of 125 to 8000 Hz for air conduction and 250 to 4000 Hz for bone conduction. In cases of significant interaural difference in hearing thresholds, cross-hearing may occur, that is, hearing the stimulus test in the untested ear. This can be avoided by applying masking noise to the untested ear (Martin & Clark, 2014).

Pure-tone audiometry is a psychoacoustic test, implying conscious collaboration of the individual under evaluation. It is not always possible to perform pure-tone audiometry in young children and uncooperative adults. Due to these limitations, we used inclusion/exclusion criteria related to age and pathological/otological history.

2.1.3 Sound-field audiometry

Sound-field audiometry was used in the first two phases of the study. It was used to test the effect of ear canal occlusion on the auditory thresholds, which required the contralateral ear masked and the use of warble tones. There are two relevant standards for sound-field audiometry [ISO 8253-2 (1998) and ISO 389-7 (2005)]. These international standards define test stimuli, calibration, equipment and procedures.

Sound-field audiometry is performed with loudspeakers, using warble tones and narrow band noise as the stimulus signal. Pure tones are not useful in sound-field audiometry, because it creates standing waves, resulting in variable results. Sound field audiometry requires more sophisticated equipment and accommodations (larger soundproof audiometric booths). Additionally, the results of sound-field audiometry can be affected by head movements of the tested individuals, because the position of the loudspeakers relative to the patient is altered (ASHA, 1991). Sound-field measurements test hearing binaurally, and the ear with better hearing dominates. Masking should be used when ears need to be tested individually in sound-field measurements (ISO, 2009).

Sound-field measurements can be prejudiced by reverberation, reflections and standing waves generated by the speakers in the room test (Laboratory, 2002), but these technical problems can be avoided or reduced using strategies to absorb theses artifacts and limiting the furniture inside the room test (ISO, 2009).

The characteristics of the soundproof room are standardized in order to enable reproducibility and comparability of the results with different equipment and settings. ISO 8253-2 provides guidelines for "quasi-free sound field conditions" so that the effect of the environment is negligible in the tested frequency range. The requirements for almost free conditions are as follows (ISO, 2009):

- The speakers are placed at ear level, at least 1 meter from the listener's head (reference point).
- On the left-right and up-down axes, the SPL produced by the speakers at positions 0.15 meter from the reference point should not deviate by more than ± 2 dB (without listener and chair)
- On the front-rear axis, the SPL produced by the speakers at positions ± 0.10 meter from the reference point should not deviate by more than ±1 dB from the theoretical value provided by the law of the inverse sound pressure distance (without listener and chair).

2.1.4 Soundproof room

All audiological tests were performed inside a soundproof room under strict and controlled conditions. Soundproof room was in accordance with to ISO 8253 and 389 standards. Audiometer calibrations were documented listing the specific functions tested and the results (see Appendix 7.7). The background noise levels in the audiometric testing room were similarly documented (see Appendix 7.7).

Clinical hearing assessments should be carried out under strict and controlled conditions, in facilities specifically designed for this purpose. Therefore, hearing assessments were performed in a controlled acoustic environment, in order to obtain the most accurate results. All audiological tests were performed according to the international standard ISO 8253. This international standard has three elements which are defined and specified as follows:

- ISO 8253-1 (Pure-tone and Narrow Band Test Signals) - The main objective of this standard is to establish the levels of hearing thresholds, mainly in the frequency range of 125 Hz to 8000 Hz. Headphones or ear inserts can be used to carry out assessments and tests in adults.
- ISO 8253-2 [Sound Field (free field) Audiometry] - This part of the standard establishes the sound field audiometry. It also focuses on pediatric assessment and the best evaluation of the functional gain achieved following the fitting of a hearing aid (or a cochlear or other auditory implant).
- ISO 8253-3 (Speech Audiometry) - This section of the standard establishes speech audiometry and is generally referred to when considering hearing impairment or audiological rehabilitation.

The permitted background noise level allowed should not exceed 57 dB (at 500 Hz), assuming that noise barrier headphones are used (audio cups) and according to ISO BS EN ISO 8253-1 (2010). The overall specifications for the audiometric test rooms environment can vary depending on:

- Size: for audiometric purposes, a test room should have a minimum area of 8 m^2 for clinical tests and assessments. A larger room implies a smaller impact from the furniture, equipment, and people on the field-free environment.

- Ambient noise levels: the guidelines recommend that ambient sound pressure levels should not exceed specific levels at certain frequencies when testing down to 250 Hz and 0 dB HL.
- Reverberation times: the reverberation times inside an audiometric test room should not exceed 0.25 seconds.
- Layout: to ensure consistent testing results the furniture and equipment should be considered inside the test room. Ideally, they should be placed in fixed positions to ensure that the acoustic measurements are not affected.
- Loudspeakers: the speakers should be positioned at the height of the seated listener's head and angled directly to the listener. Speakers must be at least 1 meter away to minimize the effects of the inverse square law.
- Doors, windows and ventilation: audiometric test rooms should have acoustic attenuating doors and windows, and ventilation systems.
- Acoustic construction: test rooms are designed and built to isolate as much as possible the room space from the building where they were inserted. Single or double walls can be installed, depending on the level of sound insulation required. A single acoustic wall is generally capable of achieving a 40dB to 45dB sound reduction. Double walls can achieve a reduction of 70 to 75dB.

The effectiveness of the approach described above can be questionable in relation to the real benefits and the cost of prefabricated sound rooms. This assessment must be made taking into account the cost-benefit, given that the level of background noise allowed is relatively high (Margolis & Madsen, 2015).

2.1.5 Hearing disorders

Patients with unilateral conductive hearing loss of several etiologies were included in this study. Some pathologies that affect the conductive system (the ME) were selected: otosclerosis, acute tympanic perforation, chronic otitis media, and otitis media with effusion. The diagnosis of conductive hearing loss was based on history, physical examination, and audiological assessments.

Otosclerosis is an osteodystrophy of labyrinth bone capsule with consequent ankylosis of the stapes in the oval window, eventually with lesion of the sensorineural elements of the cochlea. A study of 1452 human temporal bones from white subjects revealed a incidence of otosclerosis of 12.75% after exclusion of infants (Hueb, Goycoolea, Paparella, & Oliveira, 1991). Other histological study identified otosclerotic lesions in up to 12.5% of the Caucasian population, contrasting to hearing loss (clinical otosclerosis) that was detected in only 0.3-0.4% of the same population (Declau et al., 2001). When symptomatic, progressive hearing loss occurs in 70-80% of people, almost 50% of patients report tinnitus and 10% report dizziness or vertigo. The onset of the disease may range from the third to the sixth decade of life, although it is more likely to occur in the third decade. Clinically (hearing loss), women are more affected than men (1.5 to 2:1).

The confirmation of diagnosis is achieved by audiological tests. The tympanogram is usually type As (s=stiff) reflecting an ossicular mobility reduction. Accordingly, the stapedial reflex becomes progressively absent in these patients. The audiometric test is the gold standard for the diagnosis of conductive hearing loss. Schwartz's sign, a red spot behind TM, can occur in patients with active lesions of otosclerosis due to the increased vasculature. The active form of otosclerosis is better

detected on CT scans as hypodense area in the margin of the oval window or other locations of the inner ear. The definitive diagnosis of otosclerosis is obtained intraoperatively during stapes surgery.

Acute TM perforation may arise from trauma, infection, pressure, or iatrogenic causes. Trauma-associated injuries can be caused by objects such as bobby pins, cotton swabs, or other foreign bodies inserted into the ear; other situations more severe include skull fractures, acid burns, welding, or metalworking slag burns. Infection is the principal cause of TM perforations and the most common infectious cause is acute otitis media. Acute necrotic myringitis with fulminant necrosis (due to beta-hemolytic streptococcus) can lead to a perforation. Pressure-induced perforations can be caused by loud noises or explosions, changes in ambient pressure that occur during flying or underwater diving, or open palm trauma (slapping). Iatrogenic causes during medical care include inexpertly performed irrigation of the EAC for wax removal, ventilating tube insertion, and emergency myringotomy (for hyperbaric therapy).

The mechanism of sound transmission from the environment to the inner ear depends on the integrity of the TM and the pressure gradient outside and inside the TM. The existence of a TM perforation reduces the surface area of the membrane available for transmitting the sound pressure and allows to sound pass directly to ME, without the pressure gradient. As a result, the effectiveness of the TM to transmit sound to the ossicular chain is impaired, as well the level of hearing (Gan, Sun, Feng, & Wood, 2006). Therefore, the size of the perforation is directly proportional to the degree of hearing loss (Mehta, Rosowski, Voss, O'Neil, & Merchant, 2006). The location of the TM perforation is also believed to have a significant effect on the severity of hearing loss (Ahmad & Ramani, 1979). Perforations in the posterior quadrant are related to affect hearing more

than anterior perforations, due to direct exposure of the round window to sound waves. TM perforations involving the *manubrium* have more severe hearing consequences than those of comparable size in other tympanic locations (Ahmad & Ramani, 1979).

In general, otitis media can be defined as an inflammation of the ME without reference to pathogenesis or etiology (Gates et al., 2002). Several systems of nomenclature have been developed to distinguish between different types of otitis media. These systems reflect the incomplete understanding of the processes responsible for the inflammation and healing of the ME (Gates et al., 2002). For the purposes of this document, chronic suppurative otitis media (CSOM) is defined as a chronic inflammation of the ME, including tympanic and mastoid cavity, presenting recurrent ear discharges through a tympanic perforation.

CSOM is a major cause of acquired hearing impairment of varying severity, mostly in developing countries (Akinpelu et al., 2008). The prevalence of CSOM has an equitable gender distribution. The distribution of prevalence by age is unknown, but an annual incidence of 39 cases per 100,000 in children and adolescents under 15 years is reported. (Acuin, 2004).The diagnosis of CSOM is based on history and examination findings on otoscopy supplemented by culture of the ear discharge and diagnostic imaging studies of the temporal bone (Marchisio et al., 2013). Gram stains and cultures will assist in guiding therapy and are usually reserved for cases that fail standard topical therapy. Audiometry should be considered in all patients with CSOM to establish the type and degree of hearing loss. A high-resolution CT scan in both coronal and axial planes is the gold standard and is preferred to conventional radiology or magnetic resonance imaging (MRI).

Otitis media with effusion (OME) is a condition characterized by the presence of fluid in the ME, without signs or symptoms of acute ear infection. OME is one of the most common and chronic conditions of childhood (two-thirds of children had at least one episode of OME by the age of 3 years) (Monasta et al., 2012; Teele, Klein, & Rosner, 1989). OME has a lower prevalence in adults and is frequently associated with underlying conditions —particularly when unilateral. Diagnosis can be made by clinical history, otoscopic examination, and audiological evaluation. Hearing loss is usually the first and most common symptom. Older children and adults, in addition to hearing loss, usually have also fullness in the ear and tinnitus. On otoscopic examination, the TM is often dull or opaque with impaired mobility; an air-fluid level or bubbles are occasionally visible in the ME. Pneumatic otoscope and tympanometry are complementary tests, and pneumatic otoscopy is accordingly recommended as the primary test for the diagnosis of OME; tympanometry is the confirmatory test (Rovers, Schilder, Zielhuis, & Rosenfeld, 2004; Rovers & Zielhuis, 2004; Stewart, Manolidis, Wynn, & Bautista, 2001). A Type B tympanogram have a sensitivity and specificity of up to 93% for detecting OME (Dempster & MacKenzie, 1991). Further evidence is obtained with an audiogram (typically showing mild conductive hearing loss).

2.2 Ethical Decisions

The protocol for these studies was both evaluated and approved by the Institutional Review Board Institutional Review Board (CES) of the Centro Hospitalar de Lisboa Ocidental (CHLO) and the Ethics Research Committee (CEFCM) of the NOVA Medical School | Faculdade de Ciências Médicas of the Universidade Nova de Lisboa (NMS|FCM-UNL). The protocol has been also authorized by the National Commission for Data Protection (CNPD).

These studies were conducted according to the Declaration of Helsinki. The individuals who agreed to participate in the researches signed an informed consent (Appendix 7.1).

2.2.1 Institutional Review Board (CES) of the CHLO

This study was approved by the Institutional Review Board (CES) of the CHLO, on Lisbon, Portugal, 19 November 2014 (Appendix 7.2).

2.2.2 Ethics Research Committee of the NMS|FCM-UNL (CEFCM)

The Ethics Research Committee of the NMS|FCM-UNL (CEFCM) has unanimously approved the Project (no. 49/2014/CEFCM), on Lisbon, Portugal, 21 October 2016 (Appendix 7.3).

2.2.3 National Commission for Data Protection (CNPD)

Notification was done to the National Commission of Data Protection (CNPD) for processing clinical investigation data. The study was approved, in the authorization no. 10967/ 2016 on 19 October 2016. The processing of personal data was authorized for the purpose of conducting a Clinical Trial (Appendix 7.4).

The inclusion of material from different publications and book chapters in this thesis may contain similar descriptions of concepts, test procedures and findings. For instance, the working principles of COT had to be clearly delineated for each publication.

Each of the following sections are based from different publications:

1. *The section "Effects of complete occlusion of the EAC on hearing thresholds and reproducibility inter-examiner" is based on the paper of the author Reis LR, Fernandes PV, Escada P. Contralateral Occlusion Test (COT): The effect of external ear canal occlusion in hearing thresholds. Acta Otorrinolaringol Esp. 2017 Jul - Aug;68(4):197-203. doi: 10.1016/j.otorri.2016.11.011.*
2. *The section" Reproducibility with aging" is based on the paper of the author Reis LR, Castelhano L, Correia F, Escada P. Contralateral Occlusion Test (COT): The Effect of External Ear Canal Occlusion with Aging. CoDAS 2019;31(3):e20180058. doi: 10.1590/2317-1782/20192018058.*
3. *The section "Testing clinical accuracy" is based on the paper of the author Reis LR, Castelhano L, Correia F, Escada P. Contralateral occlusion test: The effect of external ear canal occlusion on predicting conductive hearing loss. Acta Otorrinolaringol Esp. 2020; 71(4):235-241. doi: https://doi.org/10.1016/j.otorri.2019.08.001.*

2.3 Effects of complete occlusion of the EAC on hearing thresholds and reproducibility inter-examiners

2.3.1 Overview

Bedside testing, otherwise referred to as near-patient or point-of-care testing, is not new and remains an integral part of clinical practice (Price, 2001; Verghese et al., 2011). Many of the early diagnostic tests are initially performed at the bedside; this practice may accelerate clinical evaluation, reduce costs, and improve decision making (St John & Price, 2013). The objective of bedside testing is to obtain a prompt result, in order to treat immediately, with the best results and minimal costs (Ehrmeyer & Laessig, 2007; Porter, 2010; St John & Price, 2013).

Tuning fork testing allows a quick, qualitative assessment of hearing and also allows for the distinction between conductive and sensorineural hearing loss (Chole & Cook, 1988; Doyle et al., 1984; Ruckenstein, 1995). The evaluation of patients with unilateral hearing loss can be promptly evaluated with Weber, Rinne and other TFTs (Browning & Swan, 1988; Browning et al., 1989; Doyle et al., 1984; Isaacson & Vora, 2003; Miltenburg, 1994; Thijs & Leffers, 1989). However, none of these tests really permits a quantitative hearing assessment.

The authors of this study designed a bedside test that allows the quantitative evaluation of hearing loss with tuning forks in the presence of unilateral conductive hearing loss (Figure 19). After the confirmation of unilateral conductive hearing loss with Weber and Rinne tests, the contralateral occlusion test (COT) is performed with total occlusion of the external auditory canal (EAC) of the contralateral ear (the non-affected ear). This will produce a hearing loss on the non-affected ear that can be higher, lower,

or similar to the affected ear. In this test, the sound of a vibrating tuning fork placed in forehead at midline will lateralize to the ear with the greater hearing loss.

Figure 19- The test is performed by placing a vibrating fork in the forehead at midline of the patient, after occluding the external auditory canal of the non-affected ear.

It is necessary to quantify the effects of EAC occlusion on hearing in order to decide which tuning fork frequency is more appropriate for quantifying the loss. EAC occlusion in COT is a simple, inexpensive, reproducible, and non-invasive method. Not investigational EAC occlusion is a common situation in daily life and can modify hearing status through interference in the bone and air conduction. It can occur in various physiological or pathological conditions, including the presence of cerumen, exostosis,

or an occlusion caused by the hearing aid mold (Bricco, 1985; Chaplin & Stewart, 1998; Chien & Lin, 2012; Kroon, Lawson, Derkay, Hoffmann, & McCook, 2002; Roland et al., 2008; Wong et al., 1999). Despite the frequency of EAC occlusion in daily life, studies about this effect on hearing thresholds have not been found in the literature. There are only two studies dealing with occlusion of the external auditory canal (Chandler, 1964; Roeser et al., 2005).

The aim of this study was to measure the effects of complete occlusion of the EAC phenomenon on hearing thresholds and to evaluate the reproducibility of the method (among each examiner) in order to apply it in the COT in normal-hearing young adults. Depending on the loss induced by this effect, we also aimed to decide the best frequency for the tuning fork in order to distinguish between mild and moderate conductive hearing loss.

2.3.2 Participants

The protocol for this study was evaluated and approved by the Institutional Review Board (CES) of the Centro Hospitalar de Lisboa Ocidental (CHLO), on 19/11/2014. The study was conducted according to the Declaration of Helsinki. The individuals who agreed to participate in the research signed an informed consent.

The study sample consisted of patients from the Otolaryngology Department of the Egas Moniz Hospital in the CHLO, who were sent to the Audiology Department for audiological evaluation. This analytical and cross-sectional study used a convenience sample composed of twenty individuals (forty ears) who fulfilled the inclusion criteria: 1) age between 20 and 30 years; 2) absence of pathological, otological history; 3) normal otoscopy; 4) normal pure-tone audiometry; 5) type A tympanogram (Jerger classification); 6) oral communication ability; and 7) a signed informed consent (signed

following clarification of the procedures). Individuals with certain conditions or test results were excluded: 1) presence of hearing loss of any degree or type in pure-tone audiometry; 2) external or ME pathology or symptomatology; 3) neurological and/or psychiatric disorders that could interfere with language; 4) serious visual changes; and 5) use of ocular prosthesis that may interfere with the audiological evaluation.

2.3.3 Procedures

All patients underwent an evaluation with a complete medical and audiological examination. The tests were conducted in a soundproof test room according to ISO 8253 and 389 using an audiometer from Madsen Electronics, model Orbiter and 922 TDH39 earphones, a noise-excluding headset ME70, and a bone conductor B-71. An audiological evaluation (immittance, tonal, and vocal audiograms) was performed, and all patients were reassessed in the office. If all of the inclusion criteria were fulfilled, a study subject was given a sound field audiometry test with warble tones presented by loudspeakers. Each ear was tested, first unoccluded and then occluded, using the standard frequencies of 250, 500, 1000, and 2000 Hz in order to determine the thresholds for each frequency. The contralateral ear was suppressed by masking at 50 dB with the handset. For the purpose of this study, occlusion was operationally defined as the complete blockage of the external auditory meatus. Implicit in this definition are the psychoacoustic and physical perceptions resulting from such conditions. The occlusion was performed by application of tragus pressure by the examiner's finger until complete occlusion of the EAC (through finger sense and asking the subject) had occurred.

Accordingly, we followed the following sequential steps:

1. Right ear (RE) uncovered, left ear (LE) with masking, and determination of the hearing thresholds at 250, 500, 1000, and 2000 Hz in the RE.

2. RE with occlusion of the EAC by finger pressure on the tragus, LE with masking and determination of the thresholds at 250, 500, 1000, and 2000 Hz in the RE.

3. In the LE, we proceeded in an identical manner to the RE, repeating steps 1 and 2.

4. Steps 1–3 were sequentially repeated by another doctor in order to study possible variability between examiners and on the part of the examiner himself.

The results for each frequency before and after occlusion and for each examiner were recorded in a table. Both examiners were right-handed.

2.3.4 Statistical analysis

The data were entered into a database, and a statistical study was performed using the Statistical Package for The Social Sciences (SPSS), 21.0 version for Windows. In the first phase, we tested the conditions for statistical tests application (normality and homoscedasticity), after which we were able to select either parametric or nonparametric tests. To evaluate the effect of occlusion of the EAC on the hearing threshold we planned to use the Student's *t*-test for paired samples and in the case of parametric tests, and in the case of nonparametric tests the Wilcoxon test was used. We applied a significance level of 0.05 (5%) with a 95% interval. We tested for statistically significant differences between unoccluded and occluded conditions, examiners, left and right ears, and gender.

2.4 Reproducibility with aging

2.4.1 Overview

Many of the early diagnostic tests are initially performed at the bedside (Price, 2001; Verghese et al., 2011). This practice may accelerate clinical evaluation, reduce costs, and improve decision making (Ehrmeyer & Laessig, 2007; Porter, 2010; St John & Price, 2013). Tuning fork testing allows a quick and qualitative assessment of hearing (Chole & Cook, 1988; Doyle et al., 1984; Ruckenstein, 1995). However, none of these tests really permits a quantitative hearing assessment.

In a previous study, we described the contralateral occlusion test (COT) (Reis, Fernandes, & Escada, 2017). We designed a bedside test that allows quantitative evaluation of hearing loss in the presence of unilateral conductive hearing loss. After the confirmation of unilateral conductive hearing loss with Weber and Rinne tests (Behn et al., 2007; Isaacson & Vora, 2003; Kelly et al., 2018; Miltenburg, 1994; Stevens & Pfannenstiel, 2015), the COT is carried out with total occlusion of the external auditory canal (EAC) of the contralateral ear (the unaffected ear). This will produce a hearing loss in the unaffected ear that can be higher, lower, or similar to that in the affected ear. In this test, the sound of a vibrating tuning fork placed in the forehead at midline will lateralize to the ear with the greater hearing loss.

Once the hearing loss produced by EAC occlusion in each frequency is established, we can decide which tuning fork is more suitable to use in the COT (Reis et al., 2017). Moreover, we can evaluate the reproducibility of that effect for several ages and several frequencies.

The aim of this study was to evaluate the effects of complete occlusion of the EAC on hearing thresholds with aging in order to decide which tuning fork is more appropriate to use for the COT in individuals of different ages.

2.4.2 Participants

This study was approved by the Institutional Review Board (CES) of the West Lisbon Hospital Centre (CHLO), Lisbon, Portugal, on 19 November 2014. The study was conducted according to the Declaration of Helsinki. All participants voluntarily signed informed consent.

The study involved patients of the Department of Otolaryngology, Egas Moniz Hospital, CHLO that underwent audiological assessments in the Department of Audiology. This analytical, cross-sectional study enrolled participants by convenience sampling. The inclusion criteria included several parameters: 1) ages within one of the three groups (20–30, 40–50, or 60–70 years); 2) absence of a pathological, otological history; 3) normal otoscopy; 4) normal pure-tone audiometry (16); 5) type A tympanogram (17); 6) oral communication ability; and 7) a signed informed consent (following clarification of the study procedures). The exclusion criteria included several parameters: 1) history of external or ME pathology or symptomatology; 2) neurological and/or psychiatric disorders that could interfere with language; and 3) serious visual changes. The study sample comprised 42 individuals (84 ears), divided into three groups: 20–30 years (21 patients, 42 ears), 40–50 years (11 patients, 22 ears), and 60–70 years (10 patients, 20 ears). None of the participants had hearing aids or formal training in pure tone audiometry.

2.4.3 Procedures

All patients underwent a comprehensive medical and audiological evaluation. All tests were conducted in a soundproof test room according to the ISO 8253 and 389 standards. The following equipment was used: the Orbiter clinical audiometer (Madsen Electronics A/C; Herley, Copenhagen, Denmark), the 922 TDH39 earphones (Telephonics; Farmingdale, NY, USA), the ME70 noise-excluding headset (Madsen Electronics A/C; Herley, Copenhagen, Denmark), and the B-71 bone conductor (Radioear Corporation; New Eagle, PA, USA). An audiological study, including admittance and tonal audiograms, was performed, and patients then underwent an office-based reassessment. If all of the inclusion criteria were fulfilled, a sound field audiometry testing was given with warble tones. Each ear was first tested as non-occluded followed by occluded, using the standard frequencies of 250, 500, 1000, and 2000 Hz in order to determine each frequency's thresholds. The contralateral ear was suppressed by masking at 50 dB with the headset. For the purpose of this study, occlusion was operationally defined as complete blockage of the external auditory meatus. Implicit in this definition are the psychoacoustic and physical perceptions resulting from such conditions. The occlusion was performed by application of tragal pressure by the examiner's finger until complete occlusion of the EAC had occurred (through finger sense and asking the subject).

Accordingly, we followed several sequential steps:

1. Right ear (RE) uncovered and masked left ear (LE) with determination of the hearing thresholds at 250, 500, 1000, and 2000 Hz in the RE.

2. RE with an EAC occlusion produced by finger pressure on the tragus and masked LE with determination of the thresholds at 250, 500, 1000, and 2000 Hz in the RE.

3. In the LE, we proceeded in an identical manner to the RE by repeating steps 1 and 2.

The results for each frequency before and after occlusion were recorded in a table. The examiner was right-handed.

2.4.4 Statistical analysis

All calculations were performed with the Statistical Package for the Social Sciences 21.0® for Windows (IBM SPSS Statistics; Armonk, NY, USA). For the 20 to 30 years group and for the total sample we tested the conditions for statistical tests application (normality and homoscedasticity) in order to choose parametric or nonparametric tests, as appropriate. In order to evaluate the effect of EAC occlusion on hearing thresholds, we used nonparametric tests (40–50 and 60–70 years groups consisting of $n < 30$ in each group). We tested for statistically significant differences between non-occluded and occluded conditions, ages, LE and RE, and gender. We computed 95% confidence intervals. $p < 0.05$ was considered statistically significant.

2.5 Testing clinical accuracy

2.5.1 Overview

Many early diagnostic tests were initially performed at the bedside (Price, 2001; Verghese et al., 2011). This practice may accelerate clinical evaluation, reduce costs, and improve decision making (Ehrmeyer & Laessig, 2007; St John & Price, 2013). The tuning fork test (TFT) for hearing loss allows a quick, qualitative assessment (Chole & Cook, 1988; Doyle et al., 1984; Ruckenstein, 1995), but does not provide a quantitative hearing measurement.

In previous studies we described the contralateral occlusion test (COT) (Reis LR, 2018; Reis et al., 2017) as a bedside diagnostic test that could be used for quantitative evaluation of unilateral conductive hearing loss. After confirming unilateral conductive hearing loss with the TFT (Behn et al., 2007; Isaacson & Vora, 2003; Kelly et al., 2018; Miltenburg, 1994; Stevens & Pfannenstiel, 2015), the COT was carried out with total occlusion of the EAC of the contralateral ear (the non-affected ear). This will produce a hearing loss of the non-affected ear that can be greater, lesser, or similar to the affected ear. In this test, the sound of a vibrating tuning fork placed in the forehead at midline will lateralize to the ear with the greater hearing loss or will not lateralize to either of the ears. In the past we quantified and standardized the effects of EAC occlusion on hearing in order to decide which tuning fork frequency was more appropriate to use (Reis et al., 2017). Furthermore, we evaluated the reproducibility of the method as performed by multiple examiners and for subjects of various ages and at several frequencies (Reis LR, 2018).

The objective of the study was to evaluate the diagnostic accuracy of the COT for assessing unilateral conductive hearing loss, as measured by the gold standard of pure

tone audiometry, and to identify the audiometric threshold at which the COT may distinguish the degree of hearing loss. This study also aimed to evaluate the factors which may have value in COT accuracy in the evaluation of hearing loss severity.

2.5.2 Participants

Institutional and university ethics approval was obtained prior to the beginning of this study. The study was approved by the Institutional Review Board (CES) of the West Lisbon Hospital Centre (CHLO), on 19 November 2014; and the Ethics Research Committee (CEFCM) of the NOVA Medical School of Universidade Nova de Lisboa (NMS-UNL), on 21 October 2016 (no. 49/2014/ CEFCM). The protocol has been also authorized by the National Commission for Data Protection (CNPD, no. 10967/ 2016). The study was conducted according to the Declaration of Helsinki. All participants voluntarily signed informed consents (following clarification of the procedures).

The study involved patients of the Department of Otolaryngology, Egas Moniz Hospital, CHLO, who were diagnosed with unilateral conductive hearing loss based on history, physical examination and audiological assessments in the Department of Audiology. This analytical, cross-sectional study enrolled participants by criteria sampling. The inclusion criteria were as follows: ages between 10 and 80 years; presence of unilateral conductive hearing loss (otosclerosis, acute tympanic perforation, chronic otitis media and otitis media with effusion); on the normal ear absence of pathological, otological history, normal otoscopy, type A tympanogram (Jerger, 1970) and normal pure-tone audiometry (Standardization, 2017) appropriate to the patient's age; and oral communication ability. The exclusion criteria were as follows: history of external or ME pathology or symptomatology on the normal ear; neurological and/or psychiatric disorders that could interfere with language; and serious visual changes. The study sample

comprised 53 individuals. Patients were instructed to remove glasses and earrings. None of the participants had hearing aids, formal training in tonal audiometry or in TFT s.

The sample population consisted of 53 subjects presenting with unilateral conductive hearing loss (23 right-sided, 30 left-sided) who met the above criteria with a mean age of 47.6±16.2 years (range 12-79 years, median of 50 years). There were 37 females (69.8 %) and 16 males (30.2 %). The age distribution is shown in Figure 20.

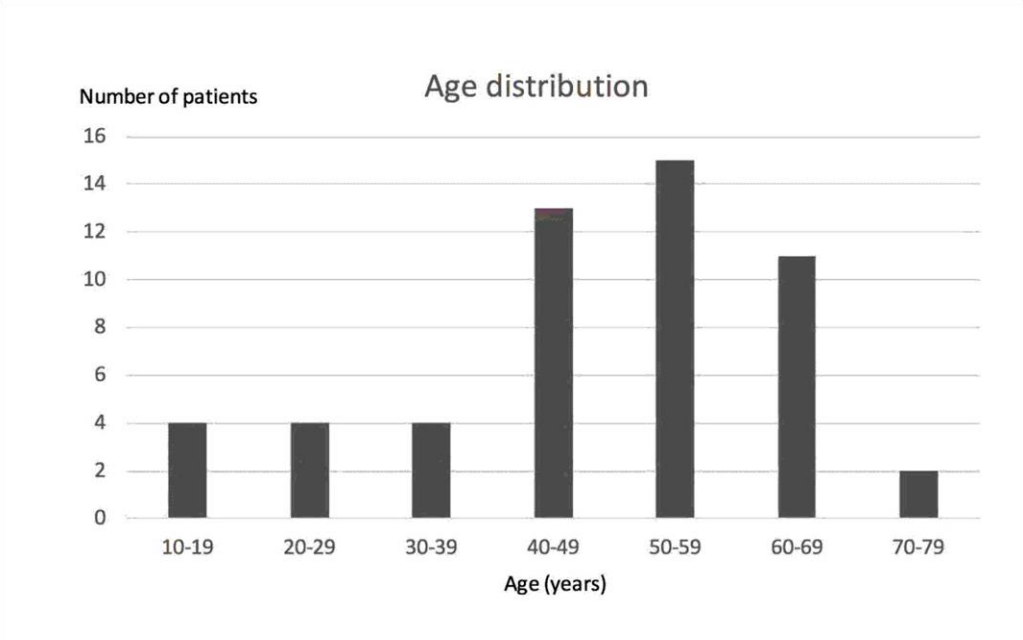

Figure 20- Age distribution of subjects for males and females combined (n = 53).

2.5.3 Procedures

All TFTs were first explained and then performed by one of the three staff otolaryngologists (L.R.R., L.C., F.C.). The physician was blinded to the audiogram test results when administering the TFTs and the audiologist was blinded to the TFT results when performing the audiogram.

All patients underwent a comprehensive medical and audiological evaluation, including admittance and pure tone audiometry. All tests were conducted in a soundproof

test room according to ISO 8253 and 389 standards. The following equipment was used: an Orbiter clinical audiometer (Madsen Electronics A/C; Herley, Copenhagen, Denmark), the 922 TDH39 earphones (Telephonics; Farmingdale, NY, USA), the ME70 noise-excluding headset (Madsen Electronics A/C; Herley, Copenhagen, Denmark), and the B-71 bone conductor (Radioear Corporation; New Eagle, PA, USA). Pure-tone measurements included air-conduction (AC) thresholds for octaves 250 to 4000 Hz and bone-conduction (BC) thresholds for octaves 500 to 4000 Hz.

If all of the inclusion criteria were fulfilled, a study subject was given a COT in a soundproof test room according the above standards. We used aluminum tuning forks at frequencies of 128 Hz, 256 Hz, 512 Hz, 1024 Hz and 2048 Hz. The presentation of each frequency was randomized, and we used a loudness comparison technique: lateralization of sound to the non-occluded ear, lateralization of sound to the occluded ear and no lateralization. The best out of three tests was considered as the definitive response. For the purpose of this study, occlusion was operationally defined as the complete blockage of the external auditory canal (EAC). Implicit in this definition are the psychoacoustic and physical perceptions resulting from such conditions. The occlusion was performed by application of tragus pressure by the examiner's finger until complete occlusion of the EAC occurred (through finger feeling and asking the subject). The examiners were right-handed.

Accordingly, we performed the following sequential steps:

1. Weber test to confirm the lateralization of sound to the affected ear. We considered lateralization positive when it occurred at three or more tuning fork frequencies.
2. Total occlusion of the EAC of the contralateral ear (the non-affected ear).
3. The base of a vibrating tuning fork was placed in the forehead at midline, equidistant from both ears, with the tines in the coronal plane and facing forward.

4. The COT was sequentially and randomly performed in a soundproof test room.

Three responses were considered: A-affected ear (lateralization of sound to the non-occluded ear), NA-non-affected ear (lateralization of sound to the occluded ear) and I-indifferent (with no lateralization). The best out of three tests was considered as the definitive response (Figure 21). For each tuning fork frequency, the COT results were recorded in a table (Appendix 7.5). The results of air-conduction (AC) and bone-conduction (BC) frequencies, for the ear with conductive hearing loss, were also recorded. The results of the COT (A, NA and I) were compared with the pure-tone average (PTA), with the ABG average (aABG) and with the gap of the 256 Hz and 512 Hz frequencies. For reporting the level of hearing function in clinical trials, we followed the standards of the Hearing Committee of the American Academy of Otolaryngology-Head and Neck Surgery: the PTA threshold was calculated by the mean of four frequencies (0.25, 0.5, 1 and 2 kHz) and the aABG threshold was calculated by the arithmetic average of the difference between the BC and AC PTAs of four frequencies (0.5, 1, 2, and 4 kHz). World Health Organization criteria were used to classify the severity of hearing loss in each ear as mild (> 25 dB through 40 dB), moderate (> 40 dB through 60 dB), severe (> 60 dB through 80 dB), or profound (> 80 dB) (WHO, 2002).

2.5.4 Statistical analysis

Statistical analysis was conducted using the Statistical Package for the Social Sciences 21.0® for Windows (IBM SPSS Statistics; Armonk, NY, USA). We calculated the PTA threshold and aABG (in dB HL) for the ear with conductive hearing loss. Our purpose was to determine if COT could be used to predict an air-conduction pure-tone threshold with different tuning forks frequencies (128 Hz, 256 Hz, 512 Hz, 1024 Hz and 2048 Hz). We tested the conditions for statistical test application (normality and

homoscedasticity) to choose parametric or nonparametric tests, as appropriate. To compare two groups, we used the Student *t* test, and for more than two groups it was used the analysis of variance (ANOVA factor 1). To test if COT could predict PTA (below or above the cut-off), a logistic regression was used. To test if COT could predict PTA (as a numerical result), a linear regression was used. We calculated the PTA cut-off value (dB HL) in the COT for each tuning fork frequency, as well as the respective sensitivity and specificity (%). We computed 95% confidence intervals and $p \leq 0.05$ was considered statistically significant.

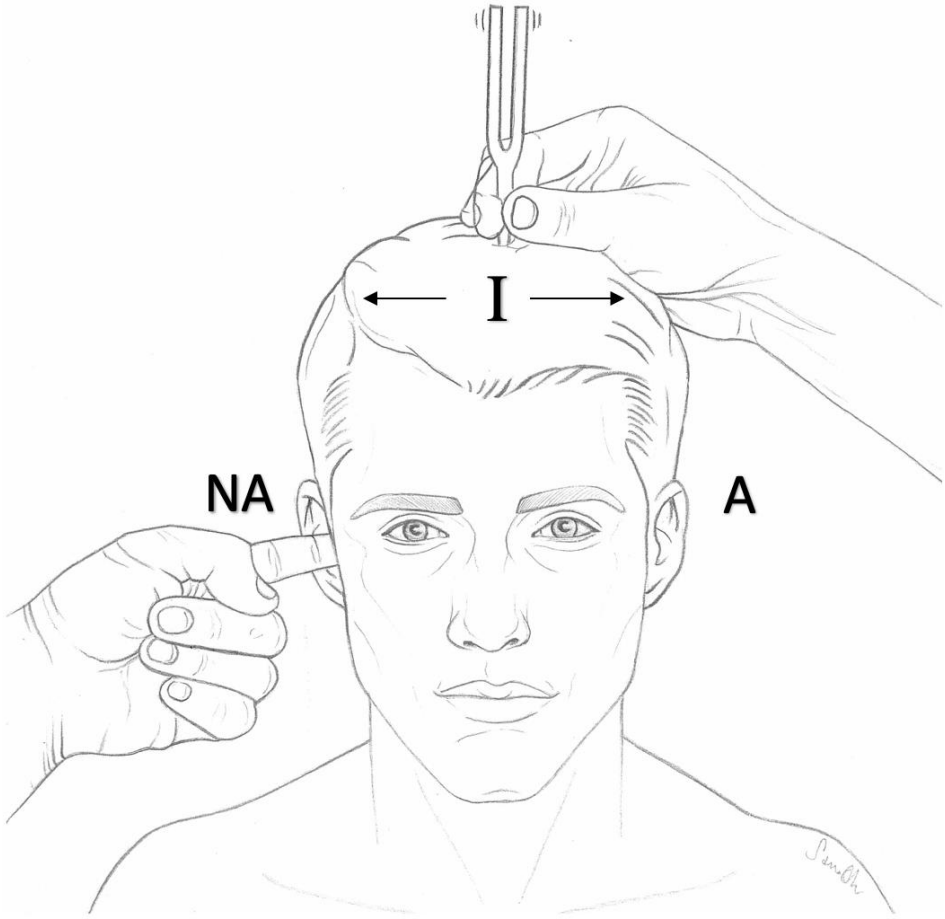

Figure 21- The sound of a vibrating tuning fork placed in forehead at midline will lateralize to the ear with the greater hearing loss or will not lateralize to either of the ears. Three responses were considered: lateralization of sound to the affected ear (A), nonaffected ear (NA) and indifferent (I).

3 RESULTS

3.1 Effects of complete occlusion of the EAC on hearing thresholds and reproducibility inter-examiners

The age of the study subjects ranged from 21 to 30 years with a mean age of 25.6 ± 3.03 years old and a median of 26 years. Nine participants were males, and eleven were females.

3.1.1 Normality testing of data

The normal distribution of data was analyzed in each dimension for the purposes of selecting either the parametric or the nonparametric tests. Table 6 shows the results obtained using the Kolmogorov-Smirnov test. All variables except the 1000 Hz With Occlusion Experimenter 2 (z = 1.384; p = .043) followed the normal distribution (p > .05), and accordingly, parametric tests were selected.

3.1.2 Differences between right and left ears

To verify if there were differences between the right and left ears, we used the Student's *t*-test (see Table 7). No statistically significant differences were found between the right and the left ears. The results demonstrated that the right ear had higher values in some dimensions (250 Hz With Occlusion Experimenter 1, 250 Hz With Occlusion Experimenter 2, 250 Hz Occlusion with average between Experimenters, 1000 Hz No Occlusion, 1000 Hz With Occlusion Experimenter 1, 1000 Hz With Occlusion Experimenter 2, 1000 Hz Occlusion with average between Experimenters, 2000 Hz No Occlusion, 2000 Hz With Occlusion Experimenter 1, 2000 Hz With Occlusion Experimenter 2 and 2000 Hz Occlusion with average between Experimenters) and the left ear in the other dimensions.

3.1.3 Differences between examiners

To verify if there were differences between examiners, we used the Student's *t*-test for two paired samples (see Table 8). The difference between examiners ranged from 0.25 dB at 2000 Hz to 1.75 dB at 500 Hz. However, there were no statistically significant differences between examiners at any of the frequencies.

Table 6- Results of normal distribution of data testing, on the several conditions (with or without occlusion, examiner 1 or examiner 2).

	z	*p*
250 Hz Without Occlusion	1.23	.097
250 Hz With Occlusion Examiner 1	1.285	.073
250 Hz With Occlusion Examiner 2	.889	.408
250 Hz With Occlusion Mean between Examiners	1.124	.160
500 Hz Without Occlusion	1.252	.087
500 Hz With Occlusion Examiner 1	1.331	.058
500 Hz With Occlusion Examiner 2	1.127	.158
500 Hz With Occlusion Mean between Examiners	.748	.630
1000 Hz Without Occlusion	1.282	.075
1000 Hz With Occlusion Examiner 1	1.034	.235
1000 Hz With Occlusion Examiner 2	1.384	.043
1000 Hz With Occlusion Mean between Examiners	.694	.721
2000 Hz Without Occlusion	1.163	.134
2000 Hz With Occlusion Examiner 1	1.213	.105
2000 Hz With Occlusion Examiner 2	1.217	.104
2000 Hz With Occlusion Mean between Examiner	.739	.646

3.1.4 Differences between unoccluded and occluded conditions

To evaluate if there were differences in hearing thresholds under different conditions, we calculated the average of the results obtained by the two examiners in the occluded condition and compared this value with the unoccluded condition. We used the Student's *t*-test for paired samples (Table 9).

Table 7- Hearing threshold levels (dB) in right and left ears, on the several conditions (with or without occlusion, examiner 1or examiner 2 and mean between examiners).

	Right Ear		Left Ear		t	p
	M	SD	M	SD		
250 Hz Without Occlusion	9.50	5.10	9.00	5.76	.291	.773
250 Hz With Occlusion Examiner 1	23.00	6.77	16.00	6.41	3.359**	.002
250 Hz With Occlusion Examiner 2	23.25	7.83	17.50	7.52	2.369*	.023
250 Hz With Occlusion Mean between Examiners	23.13	6.92	16.75	6.74	2.950**	.005
500 Hz Without Occlusion	7.50	5.00	7.75	5.25	-.154	.878
500 Hz With Occlusion Examiner 1	26.00	6.20	24.50	7.59	.684	.498
500 Hz With Occlusion Examiner 2	28.25	5.91	25.75	6.93	1.227	.227
500 Hz With Occlusion Mean between Examiners	27.13	4.89	25.13	6.10	1.145	.259
1000 Hz Without Occlusion	9.250	4.67	4.00	3.84	3.886***	<.001
1000 Hz With Occlusion Examiner 1	35.50	6.05	29.75	6.17	2.976**	.05
1000 Hz With Occlusion Examiner 2	36.25	8.25	31.25	5.59	2.243*	.031
1000 Hz With Occlusion Mean between Examiners	35.88	6.75	30.50	5.42	2.777**	.008
2000 Hz Without Occlusion	10.00	5.85	4.25	4.99	3.611***	.001
2000 Hz With Occlusion Examiner 1	42.25	4.99	36.00	5.28	3.846***	<.001
2000 Hz With Occlusion Examiner 2	42.25	6.58	36.50	6.09	2.867**	.007
2000 Hz With Occlusion Mean between Examiners	42.25	4.72	36.25	4.97	3.915***	<.001

Student's *t* test. * $p \leq .05$; ** $p \leq .01$; *** $p \leq .001$. M: mean, SD: standard deviation.

Table 8- Results in hearing thresholds (dB) with occlusion testing, depending on the frequencies (Hz) and the examiners.

	Examiner 1		Examiner 2		t	p
	M	SD	M	SD		
250 Hz With Occlusion	19.50	7.41	20.38	8.12	-1.312	.197
500 Hz With Occlusion	25.25	6.88	27.00	6.45	-1.481	.147
1000 Hz With Occlusion	32.63	6.70	33.75	7.40	-1.461	.152
2000 Hz With Occlusion	39.13	5.98	39.38	6.90	-.255	.800

Student's t-test for two paired samples. p > .05. M: mean, SD: standard deviation.

Table 9- Results in hearing thresholds (dB) depending on the frequencies (Hz), in occluded and unoccluded conditions.

	Without Occlusion		With Occlusion			
	M	SD	M	SD	T	P
250 Hz	9.25	5.38	19.94	7.48	-9.848***	<.001
500 Hz	7.63	5.06	26.13	5.55	-17.175***	<.001
1000 Hz	6.63	4.99	33.19	6.63	-38.409***	<.001
2000 Hz	7.13	5.76	39.25	5.67	-48.019***	<.001

Student's t-test for two paired samples. M: mean, SD: standard deviation.

Statistically significant differences between occluded and unoccluded conditions were found at all frequencies; for 250 Hz with t (39) = -9.848; p <0.001, for 500 Hz with t (39) = -17.175; p < .001, for 1000 Hz with t (39) = -38.409; p < .001 and for 2000 Hz with t (39) = -48.019; p < .001 were obtained. The results showed higher values with occlusion at all frequencies (Figure 22) as can be verified by the t values. As the frequency increased, the hearing threshold difference between occluded and unoccluded conditions also increased.

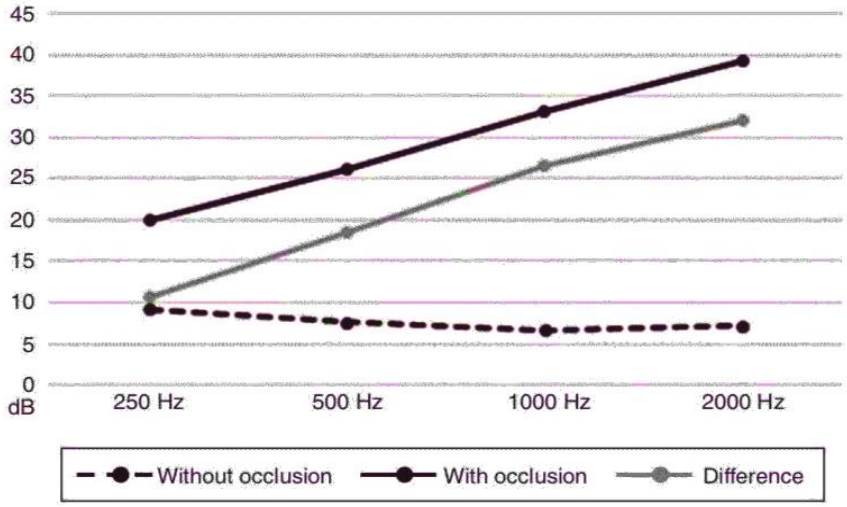

Figure 22- Evolution of hearing thresholds (dB) depending on the frequencies (Hz), in the several conditions (difference, with and without total occlusion of EAC).

There was an average hearing threshold of 7.66 dB without occlusion between 250 and 2000 Hz; with occlusion this threshold rose to 29.63 dB. Table 10 shows the values of the hearing threshold difference between occluded and unoccluded conditions at each frequency. The difference varied between 10.69 dB and 32.12 dB and increased from 250 Hz to 2000 Hz.

Table 10- Mean hearing thresholds (dB) depending on the frequencies (Hz), in the several conditions (difference, with and without total occlusion of EAC).

	250 Hz	500 Hz	1000 Hz	2000 Hz	Mean
Without Occlusion (dB)	9.25	7.63	6.63	7.13	7.66
With Occlusion (dB)	19.94	26.13	33.19	39.25	29.63
Difference (dB)	10.69	18.50	26.56	32.12	21.97

3.1.5 Differences between gender

To assess if there were gender differences, we used the *t* test for two independent samples. Statistically significant gender differences were found only at 500 Hz with

occlusion examiner 1 with t (38) = 2.202; p =.034 and at 500 Hz average experimenters with t (38) = 2.593; p = .013. The results showed higher values in males only for these two evaluations.

3.2 Reproducibility with aging

In individuals in the 20–30 years group, age ranged from 21 to 30 years (mean age, 25.6±3.03 years; median age, 26 years). In the 40–50 years group, age ranged from 41 to 50 years (mean age, 45.2±3.7 years; median age, 46 years), and in the 60–70 years group, age ranged from 60 to 67 years (mean age, 63±2.4 years; median age, 62.5 years). All the results in the different age groups are in Tables 21 to 24 (Appendix 7.8).

3.2.1 Normality testing of data

In the 20–30 years group, normal data distribution was analyzed in each dimension in order to select either the parametric or the nonparametric tests. Table 11 shows the results obtained using the Kolmogorov–Smirnov test. All variables except the 1000 Hz difference followed a normal distribution ($p < 0.05$), and accordingly, parametric tests were selected. In the others age groups with $n < 30$, normality was not tested, and nonparametric tests were used. In the total sample, only the 250 Hz with occlusion, 250 Hz difference, and average dimensions followed the normal distribution.

3.2.2 Differences between right and left ears

To compare the RE and LE in the 20–30 years group, we used the Student's t-test. In the 40–50 and 60–70 years groups, we used the Mann–Whitney test. No statistically significant differences were found between the RE and LE (Table 12).

3.2.3 Differences between occlusion and without occlusion conditions

In order to evaluate potential differences in hearing thresholds under different conditions, we compared the results under occluded condition with those in the non-occluded condition. We used the Wilcoxon test in the two upper age groups ($n > 30$), and

the paired sample t-test for the 20–30 years group (Table 13). Statistically significant differences were found between occlusion and without occlusion conditions for all measurements. Considering non-occluded and occluded conditions respectively, the average values varied for the 20– 30 years group from 7.65 dB to 29.87 dB (difference of 22.5 dB), for the 40–50 years group from 9.84 dB to 30.26 dB (difference of 20.44 dB), and for the 60–70 years group from 17.09 dB to 38.90 dB (difference of 21.84 dB). The results showed higher values with occlusion at all frequencies.

Table 11- Results of normal distribution of data in the several study conditions (with or without EAC occlusion and difference).

	z	p	z	p
	20–30 years group		Total	
250 Hz without occlusion	1.289	0.072	1.917***	0.001
250 Hz with occlusion	0.798	0.548	1.010	0.259
250 Hz difference	1.025	0.244	1.243	0.091
500 Hz without occlusion	1.328	0.059	2.023***	0.001
500 Hz with occlusion	1.204	0.110	1.650**	0.009
500 Hz difference	1.146	0.144	1.512*	0.021
1000 Hz without occlusion	1.354	0.051	2.007***	0.001
1000 Hz with occlusion	1.029	0.241	1.522*	0.019
1000 Hz difference	1.847	0.002	2.030***	0.001
2000 Hz without occlusion	1.164	0.133	1.837**	0.002
2000 Hz with occlusion	1.222	0.101	1.852**	0.002
2000 Hz difference	1.290	0.072	1.809*	0.003
Average without occlusion	0.881	0.419	1.321	0.061
Average with occlusion	0.715	0.686	0.689	0.735
Average difference	0.738	0.647	0.748	0.630

Kolmogorov–Smirnov test. *p≤0.05; **p≤0.01; ***p≤0.001.

Table 12- Hearing thresholds (dB) with occlusion in right and left ears.

Frequency	Age (years)	Right ear (dB)		Left ear (dB)		z (or t)	p
		M (dB)	SD (dB)	M (dB)	SD (dB)		
250 Hz	20–30	23.00	6.77	16.00	6.41	3.359**	.002
	40–50	17.27	8.48	17.27	8.17	0.000****	1.000
	60–70	18.00	5.87	15.00	11.06	–0.731****	.465
500 Hz	20–30	26.00	6.20	24.50	7.59	0.684*	.498
	40–50	18.18	7.17	19.09	7.69	–0.438****	.662
	60–70	19.50	4.97	18.00	7.53	–0.506****	.613
1000 Hz	20–30	35.50	6.05	29.75	6.17	2.976**	.05
	40–50	19.09	5.84	22.73	6.84	–1.079****	.281
	60–70	22.50	7.17	24.50	6.85	–0.750****	.453
2000 Hz	20–30	42.25	4.99	36.00	5.28	3.846***	<0.001
	40–50	26.82	6.43	25.00	5.00	–0.797****	.425
	60–70	28.50	6.69	28.50	10.55	–0.039****	.969

Student's t-test, and Mann–Whitney test. *p≤0.05; **p≤0.01; ***p≤0.001;
M: mean; SD: standard deviation.

3.2.4 Differences between ages

In order to test if there were differences between age groups, and because not all dimensions followed a normal distribution, a non-parametric test was chosen (Kruskal–Wallis test). Statistically significant differences were found for the three age groups and for all evaluations, except for 500 Hz difference and average difference (Table 14). For the 500 Hz difference the results ranged from 18.33 dB (40-50 years group) to 19.51 dB (20-30 years group), with an average rounded to 19 dB. In order to compare between pairs for statistically significant differences, we used the Mann-Whitney test. Comparing the 20-30 years group with the 40-50 years group we found higher values for the 20-30 years group only at 1000 Hz difference and 2000 Hz difference, and for the 40-50 years group for the remaining items (250 Hz without occlusion, 250 Hz with occlusion, 250 Hz

difference, 500 Hz with occlusion, 2000 Hz with occlusion and average without occlusion). Comparing the 20-30 years group with the 60-70 years group we found higher values in the 20-30 years group only at 2000 Hz difference, and we found higher values in the 40-50 years group for 250 Hz without occlusion, 250 Hz with occlusion, 250 Hz difference, 500 Hz without occlusion, 500 Hz with occlusion, 1000 Hz without occlusion, 1000 Hz with occlusion, 2000 Hz without occlusion, 2000 Hz with occlusion, average without occlusion and average with occlusion. Finally, comparing the 40-50 years group with the 60-70 years group we found higher values only for the 60-70 years in 250 Hz without occlusion, 250 Hz with occlusion, 500 Hz without occlusion, 500 Hz with occlusion, 1000 Hz without occlusion, 1000 Hz with occlusion, 2000 Hz without occlusion, 2000 Hz with occlusion, average without occlusion e average with occlusion. Based on Table 13, in each age group and as the frequency increased, the hearing threshold difference between occlusion and without occlusion conditions also increased (Figure 23). However, the correlation is statistically significant for the 40-50 ($r = .99$; $p = .002$) and 60-70 ($r = .99$; $p = .014$) years groups but not for the 20-30 years group ($r = .94$; $p = .059$).

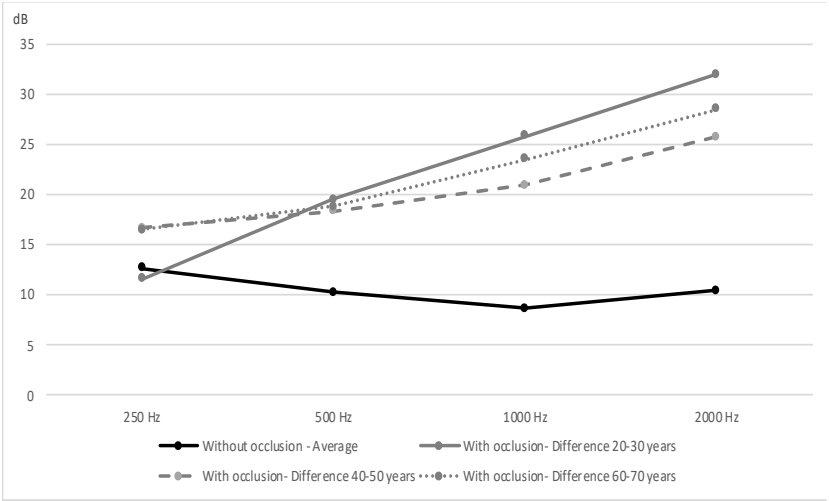

Figure 23- Occlusion/without occlusion difference (dB) and hearing average without occlusion (dB), for each frequency (250 Hz, 500 Hz, 1000 Hz and 2000 Hz) in different age groups.

Table 13- Hearing thresholds without occlusion and occlusion conditions at different frequencies and in the different age groups.

Frequency	Age (years)	Without occlusion		With occlusion		z (or t***)	P
		M (dB)	SD (dB)	M (dB)	SD (dB)		
250 Hz	20–30	9.27	5.31	20.85	8.58	–9.251***	p<0.001
	40–50	12.73	4.00	30.00	9.26	–4.124*	p<0.001
	60–70	19.25	6.94	35.75	9.22	–3.936*	p<0.001
500 Hz	20–30	7.56	5.02	27.07	6.42	–18.18***	p<0.001
	40–50	9.77	3.61	28.41	7.62	–4.130*	p<0.001
	60–70	16.00	6.20	34.75	8.19	–3.946*	p<0.001
1000 Hz	20–30	6.59	4.93	32.44	6.72	–39.71	p<0.001
	40–50	7.95	4.27	28.86	5.55	–4.132*	p<0.001
	60–70	13.50	8.29	37.00	8.01	–3.958*	p<0.001
2000 Hz	20–30	7.07	5.70	39.02	5.94	–41.04***	p<0.001
	40–50	8.18	6.08	34.09	6.10	–4.157*	p<0.001
	60–70	19.50	9.31	48.00	10.44	–3.940*	p<0.001
Average	20–30	7.65	3.81	29.87	5.09	–35.95***	p<0.001
	40–50	9.84	2.77	30.26	5.25	–4.017*	p<0.001
	60–70	17.09	6.33	38.90	6.94	–3.924*	p<-.001

Wilcoxon test, and the paired sample t-test. *p≤.05; ***p≤ .001 in 20–30 years group.
M: mean; SD: standard deviation.

3.2.5 Differences between gender

To assess if there were gender differences in the 20–30 years group, we used the t-test for two independent samples. There were no statistically significant gender differences except at 500 Hz with occlusion (t [38] = 2.202 and p = 0.034). For the 40–50 and 60–70 years groups, we used the Mann–Whitney test. Equally, for the 40–50 years group, statistically significant gender differences were observed only at 500 Hz difference, with z=–2.096 and p= 0.036. The results showed higher values in males only for these two evaluations. In the 60–70 years group, no statistically significant differences

were found between genders (Table 15).

Table 14- Hearing thresholds in the various age groups, for the different frequencies and conditions (without occlusion of the EAC, occlusion of the EAC, and difference).

	20–30 years		40–50 years		60–70 years		X^2	p
	M (dB)	SD (dB)	M (dB)	SD (dB)	M (dB)	SD (dB)		
250 Hz without occlusion	9.27	5.31	12.86	4.05	19.25	6.94	27.009***	<.001
250 Hz with occlusion	20.85	8.58	29.52	9.21	34.75	9.22	26.655***	<.001
250 Hz difference	11.59	8.02	16.67	7.80	16.50	8.75	6.700*	.035
500 Hz without occlusion	7.56	5.02	10.00	3.54	16.00	6.20	26.529***	<.001
500 Hz with occlusion	27.07	6.42	28.33	7.80	34.75	8.19	10.805**	.005
500 Hz difference	19.51	6.87	18.33	7.30	18.75	6.26	.240	.887
1000 Hz without occlusion	6.59	4.93	8.10	4.32	13.50	8.29	12.810**	.002
1000 Hz with occlusion	32.44	6.72	29.05	5.62	37.00	8.01	11.908**	.003
1000 Hz difference	25.85	4.17	20.95	6.64	23.50	6.90	8.653*	.013
2000 Hz without occlusion	7.07	5.70	8.33	6.19	19.50	9.31	23.637***	<.001
2000 Hz with occlusion	39.02	5.94	34.05	6.25	48.00	10.44	23.417***	<.001
2000 Hz difference	31.95	4.99	25.71	5.76	28.50	8.60	14.338***	.001
Average without occlusion	7.65	3.81	9.84	2.77	17.09	6.33	33.199***	<.001
Average with occlusion	29.87	5.09	30.26	5.25	38.90	6.94	22.538***	<.001
Average difference	22.25	3.96	20.44	5.28	21.84	5.00	1.496	.473

Kruskal–Wallis test. * $p \leq .05$; ** $p \leq .01$; *** $p \leq .001$. EAM: external auditory meatus.

Table 15- Differences between gender in hearing thresholds (dB) with occlusion difference.

Frequency	Age	Male		Female		z	p
	(years)	M (dB)	SD (dB)	M (dB)	SD (dB)	(or t)	
250 Hz	20–30	19.72	7.37	19.32	7.61	.169	.886
	40–50	18.75	8.82	15.50	7.25	-873	.383
	60–70	17.50	9.50	15.50	8.32	-.539	.590
500 Hz	20–30	27.78	5.75	23.18	7.33	2.202	.034
	40–50	21.67	6.16	15.00	7.07	-2.096	.036
	60–70	19.00	7.38	18.50	5.30	-.545	.586
1000 Hz	20–30	33.33	5.94	32.05	7.35	.600	.552
	40–50	21.67	6.85	20.00	6.24	-.508	.612
	60–70	24.50	5.99	22.50	7.91	-.671	.502
2000 Hz	20–30	39.17	5.75	39.09	6.29	.039	.969
	40–50	27.08	5.42	24.50	5.99	-1.079	.281
	60–70	30.00	9.13	27.00	8.23	-.271	.787

t-test for two independent samples, and Mann–Whitney test. $p \leq .05$. M: mean; SD: standard deviation.

3.3 Testing clinical accuracy

The aABG ranged from 6.25 dB to 50 dB and the PTA ranged from 21.25 dB to 88.75 dB (Table 16). To evaluate the COT results, the analysis of variance (ANOVA factor 1) was used. To test differences among pairs, we used the Student's t test (Table 17). Statistically significant differences were found for the COT with the 256 Hz tuning fork for the aABG and PTA variables. Comparing the groups, by par, and eliminating the multiple comparisons effect, there were statistically significant differences for aABG between the I and A groups, where I scores were lower than A ($p = 0.048$) and between the NA and A groups, where NA scores were lower than A ($p = 0.011$). There were also statistically significant differences for PTA between the NA and A groups, where NA scores were lower than A ($p = 0.018$), and for 250 Hz ABG between the NA and A groups, where NA scores were lower than A ($p = 0.048$). For the COT with the 512 Hz tuning fork, we found statistically significant differences for all variables. Comparing the groups, coupled and eliminating the multiple comparisons effect, there were statistically significant differences between groups A and I, where results of I were lower than A for all variables ($p <0.001$), and there were statistically significant differences among the NA and A, where NA scores were lower than A ($p <0.001$). For COT performed with the 1024 Hz tuning fork, statistically significant differences were found for the PTA, the 250 Hz ABG and the 500 Hz ABG variables. Comparing the groups, by par, and eliminating the multiple comparisons effect, there were statistically significant differences between groups I and NA, where I results were higher than NA in the PTA ($p = 0.035$) and in the 500 Hz ABG ($p = 0.031$). There were also differences between A and NA groups, where A scores were higher than NA in PTA ($p = 0.002$), 250 Hz ABG ($p = 0.025$) and 500 Hz ABG ($p = 0.015$). There were no statistically significant differences when the COT was performed with tuning forks of 128 Hz and 2048 Hz.

Table 16- Sample degree of hearing loss (average, dB HL).

ABG (dB HL)	PTA (dB HL)	Gap at 250 Hz	Gap at 500 Hz
29,3	44,6	38,1	32,1

aABG: average air-bone gap; PTA: pure tone average.

Table 17- Lateralization of the COT with different tuning fork frequencies under the defined study conditions (aABG, PTA, 250 Hz ABG and 500 Hz ABG).

	Affected ear		Indifferent		Non-affected ear		F	p
	M (dB)	SD (dB)	M (dB)	SD (dB)	M (dB)	SD (dB)		
COT 128 Hz	(n=27)		(n=19)		(n=7)			
aABG	27.04	8.44	26.47	13.82	19.64	11.38	1.298	0.282
PTA	49.72	13.50	43.42	20.12	39.12	20.45	1.433	0.248
250 Hz ABG	39.07	11.35	37.38	13.74	35.00	13.54	0.303	0.740
500 Hz ABG	32.41	9.84	33.68	17.55	26.43	14.64	0.737	0.484
COT 256 Hz	(n=32)		(n=16)		(n=5)			
aABG	28.75	9.70	23.08	12.33	16.25	9.19	3.853*	0.028
PTA	50.78	14.44	40.70	19.39	33.00	17.58	3.796*	0.029
250 Hz ABG	40.32	11.18	36.56	13.38	29.00	13.42	2.072	0.137
500 Hz ABG	34.22	11.37	30.63	16.92	23.00	13.51	1.640	0.204
COT 512 Hz	(n=37)		(n=13)		(n=3)			
aABG	30.37	9.70	14.46	5.18	19.58	7.53	16.731***	<0.001
PTA	53.75	13.70	27.02	9.35	33.75	10.00	22.964***	<0.001
250 Hz ABG	42.36	11.37	28.08	8.55	30.00	10.00	9.432***	<0.001
500 Hz ABG	37.03	12.16	19.23	8.38	26.67	12.58	12.110***	<0.001
COT 1024 Hz	(n=28)		(n=19)		(n=6)			
aABG	28.46	9.41	24.01	10.82	19.58	16.71	2.077	0.136
PTA	50.67	15.19	45.13	17.61	27.50	13.21	5.278**	0.008
250 Hz ABG	41.25	12.52	36.39	11.09	28.33	10.33	3.227*	0.048
500 Hz ABG	34.46	13.43	32.63	12.51	19.17	12.42	3.446*	0.040
COT 2048 Hz	(n=20)		(n=25)		(n=8)			
aABG	28.09	10.79	24.60	9.35	24.22	16.56	0.644	0.529
PTA	48.69	14.45	46.70	17.51	37.50	21.71	1.255	0.294
250 Hz ABG	39.75	11.18	37.92	12.85	34.38	14.25	0.536	0.588
500 Hz ABG	32.50	11.98	32.20	13.30	27.50	18.71	0.538	0.587

Student's t test. * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$. COT: contralateral occlusion test;
PTA: pure tone average; ABG: air-bone gap; aABG: average air-bone gap; M: mean; SD: standard

We calculated the PTA cut-off value (dB HL) in the COT for each tuning fork frequency, as well as the respective sensitivity and specificity (%) (Table 18). The cut-off value (dB HL) increased with increasing frequency of the tuning fork (Hz). For the COT with the 512 Hz tuning fork, the cut-off value was 35.6 dB. The sensitivity was higher with the tuning forks of 128 Hz (96.3%) and 512 Hz (94.6%). The specificity was also higher with the 512 Hz tuning fork (75%).

We calculated the aABG cut-off value (dB HL) in the COT for each fork frequency. The cut-off value (dB HL) was around 20 dB and was similar for the different frequencies of forks (Hz). The sensitivity and specificity were higher with the 512 Hz fork, 86.5% and 87.5% respectively.

Table 18- PTA and aABG cut-off, sensitivity and specificity for each COT performed with different tuning fork frequencies.

	Cut-off (dB HL)		Sensitivity (%)		Specificity (%)	
	PTA	aABG	PTA	aABG	PTA	aABG
COT 128 Hz	33.125	21.875	0.963	0.778	0.423	0.538
COT 256 Hz	38.750	21.875	0.844	0.781	0.524	0.619
COT 512 Hz	35.625	20.625	0.946	0.865	0.750	0.875
COT 1024 Hz	40.625	23.125	0.786	0.750	0.560	0.600
COT 2048 Hz	45.000	19.000	0.650	0.850	0.606	0.394

PTA: pure tone average; aABG: average air-bone gap; COT: contralateral occlusion test.

The NA and I results in the COT were grouped together and the PTA was quoted as ≥ the corresponding cut-off or < the corresponding cut-off. To test if COT could predict PTA, a logistic regression was used (Table 19). The COT was correlated with PTA in a statistically significant way, explaining the PTA variance from 20.1% (256 Hz tuning fork) to 59.3% (512 Hz tuning fork). We also determined whether the COT could predict the PTA as a numerical result (rather than as below or above the cut-off). The 128

Hz and 2048 Hz tuning forks were not able to predict the PTA; but with the 256 Hz, 512 Hz and 1024 Hz tuning forks, it was possible to predict PTA, albeit with lower percentages than the previous analysis. The positive and negative predictive values were tested for 256 Hz, 512 Hz and 1024 Hz tuning forks (Table 20). Using the 256 Hz fork, a positive predictive value of 73.0% and a negative predictive value of 68.8% was found, assuming a prevalence of 60.4%. For the 512 Hz tuning fork, we found a positive predictive value of 89.7% and a negative predictive value of 85.7%, assuming a prevalence of 69.8%. Using the 1024 Hz fork, we found lower scores, with a positive predictive value of 66.7% and a negative predictive value of 70.0% was found, assuming a prevalence of 67.4%

Table 19- Logistic regression to test if COT can predict PTA (quoted as ≥ the corresponding cut-off).

	R^2 Nagelkerre	B	Wald	p
COT 128 Hz	0.325	2.948	7.266**	0.007
COT 256 Hz	0.201	1.782	7.418**	0.006
COT 512 Hz	0.593	3.961	18.202***	<0.001
COT 1024 Hz	0.165	1.540	6.337*	0.012
COT 2048 Hz	0.081	1.050	3.179	0.075

* $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$. COT: contralateral occlusion test.

Table 20- Positive and negative predictive values of 256 Hz, 512 Hz and 1024 Hz COT.

	Sensitivity/Specificity	PPV	NPV
COT 256 Hz	0.844/0.524	0.730	0.688
COT 512 Hz	0.946/0.769	0.897	0.857
COT 1024 Hz	0.786/0.560	0.667	0.700

PPV- positive predictive value of tuning fork test for a PTA < 35.6 dB;
NPV- negative predictive value of tuning fork test for a PTA ≥ 35.6 dB;
COT: contralateral occlusion test.

4 DISCUSSION

4.1 Effects of complete occlusion of the EAC on hearing thresholds and reproducibility inter-examiners

The goal of bedside testing is to provide a prompt, accessible, and practical diagnostic instrument that accelerate the diagnosis and the treatment. Bedside testing may be used as a screening strategy for testing hearing in the office or emergency (Browning et al., 1989; Isaacson & Vora, 2003; Miltenburg, 1994; Yueh, Shapiro, MacLean, & Shekelle, 2003). While formal audiometry is preferable, it may not always be possible for reasons of expense or accessibility.

In bedside testing, tuning forks allow for the distinction between conductive and sensorineural hearing loss (Chole & Cook, 1988; Doyle et al., 1984; Ruckenstein, 1995). In some real clinical situations, we need a rapid or at least a strong idea of the severity of hearing loss such as in situations involving physical trauma of an ear, explosion, postoperative evaluation, or office visits. The immediate diagnostic information will increase safety and improve clinical outcomes.

Conductive hearing loss can be mimicked by occluding one normal ear with a finger. Total EAC occlusion of a normal ear will produce a hearing loss that can be higher, lower, or similar to the contralateral ear with conductive hearing loss. For this reason, it is very important to quantify and standardize this effect on hearing in order to determine which tuning fork frequency is more appropriate to use in COT to help quantify the loss. For example, if the sound of the tuning fork lateralizes to the affected ear (non-occluded ear), it suggests that the ear has a moderate to severe hearing loss; if the sound of the tuning fork lateralizes to the normal ear (occluded ear), it suggests that the ear has a mild hearing loss.

Complete occlusion of the EAC produced higher values in hearing thresholds in all frequencies, ranging from 19.94 dB (250 Hz) to 39.25 dB (2000Hz), with an average of 29.63 dB. As the frequency increased, the thresholds difference between unoccluded and occluded conditions became higher, ranging from 10.69 dB (250 Hz) to 32.12 dB (2000Hz) with an average of 21.97 dB. Statistically significant differences between unoccluded and occluded conditions were found in all frequencies. There were no statistically significant differences between examiners at any of the frequencies studied. Similarly, there were no statistically significant differences between ears or according to gender at the frequencies tested.

The study was performed only with normal-hearing young adults. Other age groups and any associated hearing loss were not tested. Therefore, it could not be demonstrated if the occlusion effect is cumulative with the presence of pre-existing hearing loss. Only the effects of total occlusion were studied. Partial occlusion was not tested. Both examiners were right-handed, but there were no statistically significant differences between the right and the left ear. Complete occlusion of the EAC using this method is original, especially because it can be easily applied and reproducible in the daily clinical practice.

EAC occlusion is a common situation in daily life. The prevalence of excessive cerumen varies between 5% and 10% in children or adults (Bricco, 1985; Roland et al., 2008). Prevalence of exostosis of the EAC is very high in individuals who practice water sport. It can be of varying degrees and occurs in more than two-thirds of individuals who participate in these activities (Chaplin & Stewart, 1998; Kroon et al., 2002; Wong et al., 1999). It is estimated that 3.8 million (14.2%) Americans aged > 50 and older with hearing loss use hearing aids (Chien & Lin, 2012).

There are only two studies in the literature about the effects of EAC occlusion on hearing thresholds. In both cases, the occlusion was performed with the use of inorganic materials (Chandler, 1964; Roeser et al., 2005). The use of an organic method (finger pressure) is different from previously described methods (gel and earplug) for EAC occlusion. Partial occlusion affects mainly high frequencies; thresholds < 1000 Hz are significantly affected only with total occlusion (Chandler, 1964). However, the data from Chandler referred to only two patients. Roeser et al. found the same pattern of pure tone threshold variations according to the percentage of EAC occlusion, but the study was performed with only five normal-hearing adults (Roeser et al., 2005).

The changes in hearing thresholds that occur when the EAC is completely occluded can empirically be added to pre-existing hearing loss. This effect can also occur with any configuration of hearing (Ventry, Chaiklin, & Boyle, 1961). The hearing loss pattern generated by EAC occlusion observed in this study, especially at higher frequencies, may be similar to presbycusis (Bahng & Lee, 2015; Pacala & Yueh, 2012). It should therefore result in a deficit of discrimination (Ruan, Ma, Zhang, & Yu, 2014). This is of considerable significance when an individual is being fitted for a hearing aid because any amount of hearing capacity reduction of acoustic coupling must be artificially replaced by the amplifier of the hearing aid.

The evaluation of the effects of EAC occlusion demonstrated a gradual increase in hearing thresholds from 20 to 40 dB corresponding to 250 Hz and 2000 Hz, respectively. This effect may be significant in COT because permitted the determination of the appropriate tuning fork to use for this test. It is possible to conclude that for diagnosis of mild unilateral hearing loss a 256 Hz or 512 Hz tuning fork should be used, and for moderate to severe hearing loss empirically all tuning forks could be used.

4.2 Reproducibility with aging

Bedside testing may be used as a screening procedure for testing hearing in the office or in an emergency (Kelly et al., 2018; Miltenburg, 1994; Yueh et al., 2003). While formal audiometry is preferable, it may not always be possible for reasons of expense or accessibility. Tuning forks allow for the distinction between conductive and sensorineural hearing loss (Chole & Cook, 1988; Doyle et al., 1984; Ruckenstein, 1995). However, in some real clinical situations, we need a rapid or at least a strong indication of hearing loss severity. This information allows an immediate diagnosis that increase safety and improve clinical outcomes.

COT may help to quantify the hearing loss. Total EAC occlusion of a normal ear can produce a hearing loss (Chandler, 1964; Roeser et al., 2005) that can be higher, lower, or similar to the contralateral ear with conductive hearing loss. If the sound of the tuning fork lateralizes to the affected ear (non-occluded ear), it suggests that the ear has a moderate or severe hearing loss; if the sound of the tuning fork lateralizes to the normal ear (occluded ear), it suggests that the ear has a mild hearing loss.

In a prior study, the reproducibility of hearing loss induced by the EAC occlusion (between examiners) and the correlation of degree of hearing loss with frequencies were demonstrated (Reis et al., 2017). In this study the objective was to understand if the occlusion effect was reproducible with aging. At each frequency, hearing thresholds increased in the two conditions (occlusion and without occlusion) with aging; probably in relation to the normal process of hearing loss with aging. Complete EAC occlusion produced higher values for hearing thresholds in all frequencies, which increased with increasing frequencies. Differences between occluded and non-occluded conditions also increased with increasing frequencies and aging, ranging from 11.6 dB (250 Hz, 20–30

years group) to 32 dB (2000 Hz, 20–30 years group). These difference increases were homogeneous and similar with aging. However, at 500 Hz only, there were no statistically significant differences corresponding to age. The mean hearing loss produced by EAC occlusion at 500 Hz was approximately 19 dB (Table 13). There were no statistically significant differences between ears or according to gender at all frequencies tested.

In this study, our aim was to evaluate the hearing loss produced by EAC occlusion in different frequencies and find one frequency where a similar hearing loss was produced at all ages. Our study suggests that, at 500Hz, there will be a similar loss (19 dB) for all age groups. Extrapolating to the tuning fork bedside test, the 512Hz will be the ideal to be used. Thus, when performing COT with 512 Hz, we will know that occlusion of the "contralateral ear" (healthy ear) produces a 19 dB loss. Using a loudness comparison technique, we can compare the "contralateral ear" with the conductive hearing loss ear.

This study has limitations. It was performed only with normal hearing individuals. We intent to evaluate the effects of the EAC occlusion in a normal ear and extrapolate the results to the "contralateral ear" of the COT. It was not studied if the occlusion effect is cumulative with the presence of pre-existing hearing loss. Only the effects of total occlusion were studied. The examiner was right-handed, but there were no statistically significant differences between the RE and LE. The start order was not randomly performed, on the right or left side, because that was tested on a prior study.

There are only two studies about the effects of EAC occlusion on hearing thresholds (Chandler, 1964; Roeser et al., 2005). In both cases, the occlusion was performed with the use of inorganic materials (gel and earplug). The use of an organic method (finger pressure) is different from previously described methods (gel and earplug) for EAC occlusion. EAC occlusion is a common situation in daily life and includes

situations such as excessive cerumen (5% to 10% in children or adults), exostosis, and hearing aid use (14.2% of Americans aged ≥50) (Bricco, 1985; Chaplin & Stewart, 1998; Kroon et al., 2002; Roland et al., 2008; Wong et al., 1999).

TFTs can be performed with different frequencies. For routine clinical practice, tuning forks of 256 or 512 Hz are the preferred. Forks with lower frequencies produce a sense of bone vibration while those of higher frequencies have a shorter decay time. The results of our study suggest the use of a 512 Hz tuning fork for COT.

4.3 Testing clinical accuracy

The TFT allows discrimination between conductive and sensorineural hearing loss (Chole & Cook, 1988; Doyle et al., 1984; Ruckenstein, 1995). While formal audiometry is preferable, it may not always be possible for reasons of expense or accessibility. However, in some real clinical situations, we need a fast and reasonably accurate idea of the degree of hearing loss. COT may be useful in quantifying unilateral conductive hearing loss. This test can be performed at a person's bedside in a hospital or emergency room, or in a doctor's office, in situations such as traumatic perforation of TM. Since conditions presenting conductive hearing loss are often difficult to diagnose based only on clinical evaluation, referral for otolaryngologic examination and hearing tests is often to late for prompt intervention. This study was realized to explore the value of COT to quantify unilateral conductive hearing loss severity.

Using the 512 Hz tuning fork, we obtained statistically significant results in all variables with greater significance ($p<0.001$). The results with PTA were more significant than with ABG. Grouping the NA and I results, 512Hz was also the frequency that gave the greatest correlation in predicting the degree of conductive hearing loss. The sensitivity of the 512 Hz fork COT in detecting a PTA of at least 35.6 dB was 94.6% and the specificity was 75.0% for a positive predictive value of 89.7% and a negative predictive value of 85.7%, assuming a prevalence of 69.8%. Using the COT with the 512 Hz tuning fork, we correctly detected 59.3% of ears with PTA over 35.6 dB. Therefore, if a lateralization to the affected ear occurs, it is almost certain evidence of a moderate to severe conductive hearing loss.

There are various limitations that may affect the sound conduction, from the tuning fork to the ear (as the optimum affordable pressure on the skull and the amount of

pressure to apply on the external auditory meatus). During our examinations, we practiced with subjects to achieve consistent results. The etiology of the unilateral conductive hearing loss was variable and was also a limitation of our study. There is a substantial history of assessing the utility of TFT in conductive hearing loss (Browning & Swan, 1988; Chole & Cook, 1988; Doyle et al., 1984; Kelly et al., 2018; Miltenburg, 1994). Outcoming data increased the TFT reliability, considering different performance parameters. There are measurable differences in the results when using forks made of different materials (MacKechnie et al., 2013; White, 1974; Yuksel & Kemaloglu, 2017) and there are also recommendations about how to strike the tuning fork, in order to minimize overtones (Samuel & Eitelberg, 1989; Stevens & Pfannenstiel, 2015). The orientation of the tuning fork tines affects the amplitude of the sound signal at the ear (Butskiy et al., 2016; Lin et al., 2014). TFT involving children showed them to be of controversial value (Capper, Slack, & Maw, 1987; Yung & Morris, 1981). The majority of TFT studies evaluate the ABG rather than the PTA. The need for masking in audiometry is recognized but the need for masking in tuning fork testing has not been established (Burkey, Lippy, Schuring, & Rizer, 1998; Miltenburg, 1994).

There are remarkable advantages of tuning forks, such as low cost, low maintenance, and no calibration requirement. The small size allows tuning forks to be carried and handled by primary care physicians or health workers in daily practice. These simpler tests can be used in place of the audiometry gold standard as long as it is kept in mind that the results have some risk of incorrect diagnosis. This risk is justified by the simplicity, safety and convenience of the COT. The high reliability of the COT when it lateralized towards the suspect ear confirms that the test has value in the clinical setting when assessing a patient presenting with moderate or severe unilateral conductive hearing loss.

4.4 Future work

The studies presented in this thesis described the invention and validation of a bedside tuning fork test. The work related to a diagnostic test does not necessarily end with clinical validation. As we see with other TFTs, over the years, multiple studies have emerged evaluating different aspects of these tests. Therefore, future work can improve the clinical results, as well some aspects related to its performance.

An achievable goal is the construction of a real ear model with total EAC occlusion with an insertion plug and with an in-ear microphone. This will allow to measure more accurately the attenuation produced by the EAC occlusion. These measurements can also be performed at different frequencies.

Another ear model which can be build is a finite volume method. This model represents a three-dimensional numerical (virtual) reconstruction of real structures, such as ear. The sound dynamics event can be studied on each element separately and analyzed together, in order to approximate the model with the real structures.

There are several study possibilities to analyze the results obtained with different performance parameters that can increase the test's reliability. These aspects may include the tuning fork positioning, the way to strike the fork, the EAC occlusion method and the use of forks made of different materials. Finally, it is still possible to study the accuracy of the test in a larger sample.

5 CONCLUSIONS

Bedside testing brings advanced diagnostics to the clinical evaluation of individuals with hearing loss. The studies presented in this thesis described the invention and validation of a bedside tuning fork test, capable of quantitatively evaluate patients with unilateral conductive hearing loss, distinguishing mild from moderate to severe unilateral conductive hearing losses.

The novel test was named Contralateral Occlusion Test (COT) and should be added to the other tuning fork tests usually performed in the clinical evaluations of patients with hearing loss, the Weber test and the Rinne test.

Other conclusions of our studies are presented:

1. The COT should be performed with total occlusion of the EAC of the contralateral ear (the non-affected ear).
2. If the sound of the tuning fork lateralizes to the affected ear (non-occluded ear), it indicates that the ear has a moderate to severe hearing loss; if the sound of the tuning fork lateralizes to the normal ear (occluded ear) or doesn't lateralize, it indicates that the affected ear has a mild hearing loss.
3. The occlusion method as performed with the finger is reproducible, even with different examiners, with aging, tuning forks of different frequencies, right and left ears and gender of the tested subjects.
4. The hearing thresholds of the occluded ear increased for all frequencies, especially on high frequencies. The human ear is more sensitive to low frequencies, even when fully occluded.

5. Differences between non-occluded and occluded conditions were found in all frequencies. As the frequency increased, the thresholds difference between non-occluded and occluded conditions became higher.
6. The occlusion effect was reproducible with aging. At each frequency, hearing thresholds increased in the two conditions (occlusion and non-occlusion) with aging. Differences between occlusion and non-occlusion conditions also increased with increasing frequencies and aging.
7. COT can be performed with different tuning fork frequencies. For routine clinical practice, tuning forks of 256 or 512 Hz are adequate, but the 512Hz fork will be the ideal.
8. The sensitivity of the 512 Hz fork in detecting a PTA of at least 35.6 dB was 94.6% and the specificity was 75.0% for a positive predictive value of 89.7% and a negative predictive value of 85.7%, assuming a pretest prevalence of 69.8%.
9. The COT identified with high accuracy those ears with a 35.6 dB or greater PTA. If lateralization to the affected ear occurs, it is almost certain evidence that the patient has a moderate to severe conductive hearing loss in the affected ear.
10. The COT requires a skilled examiner and a basic understanding of the patho-physiology of hearing and hearing loss. The COT can allow the quantitative evaluation of hearing loss in the presence of unilateral conductive hearing loss and should be used in clinical routine practice.

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7 APPENDIX

7.1

Serviço de Otorrinolaringologia
S. Universitário, polo do H. Egas Moniz
Director Prof. Doutor Pedro Escada

Hospital de Egas Moniz

Consentimento Informado

Para realização de exames para estudo do efeito do fenómeno de oclusão do canal auditivo externo nos limiares auditivos e da sua aplicabilidade clinica na validação dum novo teste de acumetria instrumental. / Effect of ear canal occlusion phenomenon on pure-tone thresholds and its clinical applicability in validation of a new test of acumetry.

Por favor, leia com atenção todas as indicações constantes neste documento. Não hesite em solicitar mais informações ao seu médico assistente, se não estiver completamente esclarecido.

Os exames a que vai ser submetido – acumetria instrumental, audiograma tonal, audiograma vocal e impedanciometria – estão incluídos num estudo científico que visa a determinação do efeito do fenómeno de oclusão do canal auditivo externo nos limiares auditivos, para desenvolver e validar um novo teste complementar de acumetria instrumental e avaliar a eficácia do mesmo na categorização do grau de hipoacusia de transmissão unilateral induzido por diversas patologias.

O único centro envolvido é o Hospital de Egas Moniz, em Lisboa, do Centro Hospitalar de Lisboa Ocidental.

Os exames complementares de diagnóstico referidos são seguros, sem riscos, que não utilizam radiações ou administração de fármacos e são utilizados há muitos anos no diagnóstico de doenças do sistema auditivo.

Poderá interromper os exames na altura que pretender, mesmo durante a realização dos mesmos e após a sua realização poderá regressar ao seu domicílio sem nenhuns cuidados particulares. O resultado poderá ser enviado directamente ao seu médico assistente.

1 de 3

Todo este estudo é apenas dirigido para avaliar experimentalmente o efeito da oclusão total do canal auditivo externo e perceber se existe um método mais simples e rápido de perceber a gravidade da surdez de condução unilateral em diferentes doenças.

Não existe alternativa, actualmente, a este meio de avaliação clínica da acuidade auditiva para categorizar a gravidade da surdez. A alternativa é a realização de exames complementares de diagnóstico que irão ser realizados para validação do teste proposto.

No grupo dos pacientes com surdez de condução unilateral, o tratamento dos resultados dos exames que se irão realizar em nada modificarão o procedimento cirúrgico a que eventualmente possa vir a ser submetido.

Os exames que realizará ficarão arquivados no processo clínico do Hospital de Egas Moniz, em Lisboa. Poderá, se assim o entender, ter acesso a uma cópia para o seu médico assistente. A confidencialidade dos exames que irá realizar é garantida por todos os profissionais médicos e não médicos envolvidos e está contemplada pelo segredo médico.

Não há pagamento de deslocações ou contrapartidas, sendo que a participação é de caráter voluntário e a recusa em participar não acarreta prejuízos, assistenciais ou outros. (O estudo mereceu parecer favorável da Comissão de Ética)

Se autorizar, a equipa médica responsável pelo estudo científico poderá utilizar os resultados dos exames apenas para fins científicos, mantendo sempre o anonimato, apagando a identidade do doente e substituindo a mesma por um número.

Identificação do Doente

Nome: _____

Data de Nascimento: __ / ___ / 19___

Número do Processo Clínico: _____

A preencher pelo médico

Diagnóstico do Doente: _____

Confirmo que expliquei ao doente ___ , aos pais ___ ou ao seu representante legal ___, de forma adequada e inteligível, a condição clínica do doente, os exames propostos, os potenciais benefícios e prejuízos, as alternativas diagnósticas/terapêuticas possíveis, a previsibilidade de êxito da sua aplicação e possíveis resultados da realização dos exames.

Confirmo, ainda, que expliquei que os exames a realizar são exames seguros e standard no meio médico, complementados com uma avaliação clínica. Este teste complementar de diagnóstico não implica ou envolve qualquer estudo ou fator experimental que possa prejudicar a condição ou saúde do doente, antes ou posteriormente a uma eventual cirurgia.

Assinatura_____
Data __ / ___ / 201___ Número da Cédula profissional _____

A preencher pelo doente

Eu, abaixo assinado, declaro que:

Recebi e compreendi a informação que me foi prestada pelo médico acima identificado, necessária para formar devidamente a minha vontade e que concordo com o que me foi proposto e explicado pelo médico que assina este documento, tendo tomado esta decisão livremente.

Tenho conhecimento que o consentimento agora prestado pode ser livremente revogado a todo o momento, por qualquer modo ou forma, devendo essa revogação ser inequivocamente efectuada.

Autorizo a realização dos actos médicos indicados – acumetria instrumental, audiograma tonal, audiograma vocal e impedanciometria - bem como os procedimentos adicionais que sejam necessários no meu próprio interesse e justificados por razões clínicas.

Autorizo a utilização parcial ___ ou total ___ dos resultados do meu exame apenas para fins científicos, respeitando os critérios previstos para tal fim.

Assinatura _____
Data __ / ___ / 201___

No caso de não ser o doente:

Nome_____ Data __ / ___ / 201___
Morada _____
Documento de Identificação _____ de _____
Grau de Parentesco / Título de Representação _____

ESTE DOCUMENTO É COMPOSTO DE 3 PÁGINA/S E FEITO EM DUPLICADO: UMA VIA PARA O/A INVESTIGADOR/A, OUTRA PARA A PESSOA QUE CONSENTE

Rua da Junqueira, 126 1349-019 LISBOA
Telefones: 210432115/16/17/18 – 210432123/24 Fax: 210432130
E-mail: otorrinolaringologia@hegasmoniz.min-saude.pt

7.2

PARECER DA COMISSÃO DE ÉTICA PARA A SAÚDE

Projeto de Doutoramento,

"O efeito do fenómeno de oclusão do canal auditivo externo nos limiares auditivos e a sua aplicabilidade clínica na validação dum novo teste de acumetria instrumental"

Após reunião de 17 de novembro de 2014 e estando atualmente o projeto de acordo com as normas de submissão impostas por esta CES, deliberou-se emitir **parecer favorável** à realização do mesmo.

A Comissão de Ética para a Saúde solicita ao Investigador Principal que, quando da conclusão deste projeto, lhe seja enviada uma síntese dos resultados e conclusões do mesmo.

Ouvido o Relator, o processo foi votado pelos Membros da Comissão de Ética para a Saúde do Centro Hospitalar de Lisboa Ocidental presentes em reunião de 17 de novembro de 2014:

Presidente: Prof.ª Doutora Maria Teresa Marques

Dr. Carlos Costa, Enf.ª Clara Carvalho, Dra. Helena Farinha,

Dr. José Santana Carlos, Dra. Paula Peixe, Dr. Rui Teles e Padre João Valente

Pelo exposto, emitiu-se a 19 de novembro de 2014, **parecer favorável**.

Presidente da Comissão de Ética para a Saúde

Prof.ª Doutora Maria Teresa Marques

MARIA TERESA MARQUES
Presidente da Comissão
de Ética para a Saúde

Hospital de Egas Moniz
Rua de Junqueira, 126 - 1349-019 Lisboa // Telefone: 210 432 643

7.3

Decisão final sobre o projeto "O efeito do fenómeno de oclusão do canal auditivo externo nos limiares auditivos e a sua aplicabilidade clinica na validação dum novo teste de acumetria instrumental"

A Comissão de Ética da NMS|FCM-UNL (CEFCM) decidiu, por unanimidade, aprovar o projeto de investigação intitulado "O efeito do fenómeno de oclusão do canal auditivo externo nos limiares auditivos e a sua aplicabilidade clinica na validação dum novo teste de acumetria instrumental" (nº49/2014/CEFCM), submetido pelo Dr. Luis Roque dos Reis.

Lisboa, 21 de Outubro de 2016

O Presidente da Comissão de Ética,

(Prof. Doutor Diogo Pais)

TO WHOM IT MAY CONCERN

The Ethics Research Committee NMS|FCM-UNL (CEFCM) has unanimously approved the Project entitled " O efeito do fenómeno de oclusão do canal auditivo externo nos limiares auditivos e a sua aplicabilidade clinica na validação dum novo teste de acumetria instrumental " (nr.49/2014/CEFCM), submitted by Dr. Luis Roque dos Reis.

Lisbon, October 21th, 2016

The Chairman of the Ethics Research Committee,

(Diogo Pais, MD, PhD)

7.4

N/Ref. 02.02
Proc. n.º 16337 / 2016
Of. nº 31684
Data: 2016-10-19

Assunto: Notificação de tratamento de dados de investigação clínica

Com referência ao assunto em epígrafe, ficam V. Exas. notificados de todo o conteúdo da decisão desta CNPD nº 10967/ 2016 proferido em 19-10-2016, cuja cópia se anexa.

Com os melhores cumprimentos.

A Secretária da CNPD

(Isabel Cristina Cruz)

Autorização n.º 10967/ 2016

Luis Miguel Roque dos Reis notificou à Comissão Nacional de Protecção de Dados (CNPD) um tratamento de dados pessoais com a finalidade de realizar um Ensaio Clínico, denominado O efeito do fenómeno de oclusão do canal auditivo externo nos limiares auditivos e a sua aplicabilidade clinica na validação dum novo teste de acumetria instrumental. / Effect of ear canal occlusion phenomenon on pure-tone thresholds and its clinical applicability in validation of a new test of acumetry. .

O participante é identificado por um código especificamente criado para este estudo, constituído de modo a não permitir a imediata identificação do titular dos dados; designadamente, não são utilizados códigos que coincidam com os números de identificação, iniciais do nome, data de nascimento, número de telefone, ou resultem de uma composição simples desse tipo de dados. A chave da codificação só é conhecida do(s) investigador(es).

É recolhido o consentimento expresso do participante ou do seu representante legal.

A informação é recolhida diretamente do titular.

As eventuais transmissões de informação são efetuadas por referência ao código do participante, sendo, nessa medida, anónimas para o destinatário.

A CNPD já se pronunciou na Deliberação n.º 1704/2015 sobre o enquadramento legal, os fundamentos de legitimidade, os princípios aplicáveis para o correto cumprimento da Lei n.º 67/98, de 26 de outubro, alterada pela Lei n.º 103/2015, de 24 de agosto, doravante LPD, bem como sobre as condições e limites aplicáveis ao tratamento de dados efetuados para a finalidade de investigação clínica.

No caso em apreço, o tratamento objeto da notificação enquadra-se no âmbito daquela deliberação e o responsável declara expressamente que cumpre os limites e condições aplicáveis por força da LPD e da Lei n.º 21/2014, de 16 de abril, alterada pela Lei n.º 73/2015, de 27 de junho – Lei da Investigação Clínica –, explicitados na Deliberação n.º 1704/2015.

O fundamento de legitimidade é o consentimento do titular.

A informação tratada é recolhida de forma lícita, para finalidade determinada, explícita e legitima e não é excessiva – cf. alíneas a), b) e c) do n.º 1 do artigo 5.º da LPD.

Assim, nos termos das disposições conjugadas do n.º 2 do artigo 7.º, da alínea a) do n.º 1 do artigo 28.º e do artigo 30.º da LPD, bem como do n.º 3 do artigo 1.º e do n.º 9 do artigo 16.º ambos da Lei de Investigação Clínica, com as condições e limites explicitados na Deliberação da CNPD n.º 1704/2015, que aqui se dão por reproduzidos, autoriza-se o presente tratamento de dados pessoais nos seguintes termos:

Responsável – Luis Miguel Roque dos Reis

Finalidade – Ensaio Clínico, denominado O efeito do fenómeno de oclusão do canal auditivo externo nos limiares auditivos e a sua aplicabilidade clinica na validação dum novo teste de acumetria instrumental. / Effect of ear canal occlusion phenomenon on pure-tone thresholds and its clinical applicability in validation of a new test of acumetry.

Categoria de dados pessoais tratados – Código do participante; dados da história clínica; dados dados de exame físico

Exercício do direito de acesso – Através dos investigadores, presencialmente

Comunicações, interconexões e fluxos transfronteiriços de dados pessoais identificáveis no destinatário – Não existem

Prazo máximo de conservação dos dados – A chave que produziu o código que permite a identificação indireta do titular dos dados deve ser eliminada nos prazos previstos no ponto 5.2 do Anexo I do Decreto-Lei n.º 176/2006, de 30 de agosto, alterado, por último, pelo Decreto-Lei n.º 51/2014, de 25 de agosto.

Da LPD e da Lei de Investigação Clínica, nos termos e condições fixados na presente Autorização e desenvolvidos na Deliberação da CNPD n.º 1704/2015, resultam obrigações que o responsável tem de cumprir. Destas deve dar conhecimento a todos os que intervenham no tratamento de dados pessoais.

Lisboa, 19-10-2016

A Presidente

Filipa Calvão

7.5

Tabela - 3ª fase do estudo

Identificação	
data	___ / ___ / 201__
nome	
idade	___ anos
género	masculino / feminino
nº processo	
contacto	

Dados clínicos	
diagnóstico	Otosclerose / OM crónica / OM com derrame / perf timpânica
lado afetado	OD / OE
otoscopia	Ouvido com surdez ....
weber	direita / esquerda
COT	Tabela em anexo
aud tonal	Anexar cópia

Diapasão (Hz)					
COT	**128**	**256**	**512**	**1024**	**2048**
Sala de consulta					
Cabine insonorizada					

Diapasão (Hz)					
WEBER	**128**	**256**	**512**	**1024**	**2048**
Sala de consulta					
Cabine insonorizada					

7.6

GN Otometrics A/S CALIBRATION CERTIFICATE

Calibration chart for : ME 70

Tone Norm : ISO 389 Instrument : OB922 Date : 10-Okt-2007
NBN Norm : Serial no : 277525 ID : WP

Freq Hz	Tone Norm	Mic/ Mast Corr.	Att. Pos.	Target Level *1/*2	Left output	Right output	NBN Norm	Target Level *3	Left output	Right output
125	45.00			80	80.3	79.6	4.00	80	80.2	80.2
250	25.50			80	80.0	80.1	4.00	80	80.3	80.3
500	11.50			80	79.5	79.6	4.00	80	80.3	80.5
750	7.50			80	79.7	79.7	5.00	80	80.1	79.8
1000	7.00			80	80.0	80.0	6.00	80	80.1	80.3
1500	6.50			80	79.8	79.6	6.00	80	80.4	79.6
2000	9.00			80	80.3	80.0	6.00	80	80.0	80.2
3000	10.00			80	79.6	79.7	6.00	80	79.8	80.3
4000	9.50			80	79.6	80.2	5.00	80	79.5	80.2
6000	15.50			80	79.8	80.4	5.00	80	80.5	80.1
8000	13.00			80	80.0	79.5	5.00	80	79.7	79.5
10000	13.00			80	80.4	79.7	5.00	80	79.9	79.7
12500	13.00			80	80.3	79.7	5.00	80	80.3	79.9
14000	13.00			80	NA	NA	5.00	80	NA	NA
16000	13.00			80	NA	NA	5.00	80	NA	NA

Ext Norm :

Input	Ext Norm	Mic Corr. *7	Att. Pos.	Target Level *4/*5	Left output	Right output
WNoise	0.00			80	80.1	80.1
SNoise	25.00			80	80.4	80.4
CD1 *6/*8	22.00			80	80.0	80.0
CD2 *6/*8	22.00			80	80.0	80.0
Mic *6/*8	22.00			80	80.0	80.0

*? Remarks is placed on bone calibration chart

Headband pressure :

Target	Value
9,5+/- 1,0N	10.2N

GN Otometrics A/S CALIBRATION CERTIFICATE

Instrument : OB922 Transducer calibrated (X) :

Serial no : 277525 HL Calibrated (X) : X

Calibration Norm : ME ISO NORM

ME 70	serial no	: Left :	343849	Right :	346274
	serial no	: Left :		Right :	
	serial no	: Left :		Right :	
	serial no	: Left :		Right :	
	serial no	:			
B 71	serial no	:	277525		

B&K Equipment		Type:	Serial no:
Amplifier	:	GRAS 12A	15355
Microphone Left 1"	:	40 EN	72286
Microphone Right 1"	:	40EN	52835
Microphone Left 1/2"	:	40 AG	70834
Microphone Right 1/2"	:	40 AG	24204
Microphone Left 1"	:		
Microphone Right 1"	:		
Ear Left 1"	:	B&K 4152	1863552
Ear Right 1"	:	B&K 4152	0745342
Ear Left 1/2"	:	B&K 4153	-------
Ear Right 1/2"	:	B&K 4153	-------
Ear Left 1"	:		
Ear Right 1"	:		
Preamplifier Left 1"	:	B&K 2669	2152420
Preamplifier Right 1"	:	B&K 2669	2223003
Preamplifier Left 1/2"	:	B&K 2669	2299359
Preamplifier Right 1/2"	:	B&K 2669	2083924
Preamplifier Left 1"	:		
Preamplifier Right 1"	:		
Calibrator	:	B&K 4231	2309018
Mastoid	:	4930	591044
Counter	:	Kontron	25793

D.: 10-Okt-2007 ID.: WP

form nr :

WIDEX
high definition hearing

FOLHA DE VERIFICAÇÃO DE CALIBRAÇÃO DE EQUIPAMENTO AUDIOLÓGICO

MARCA MADSEN
MODELO OB822 Nº SÉRIE 277525
DATA DA VERIFICAÇÃO 8.4.2011
PRÓXIMA VERIFICAÇÃO 8.4.2012

	MARCA	MODELO	Nº SÉRIE
SONÓMETRO	BRUEL & KJAER	2235	1484296
MASTOIDE	BRUEL & KJAER	4930	1727455
OUVIDO ARTIFICIAL	BRUEL & KJAER	4152	1756494

ACESSÓRIOS VERIFICADOS

AUSCULTADORES TDH39 ☐
AUSCULTADORES ME70 ☐
AUSC. SENNHEISER HDA200 ☐
AUSC. IPSI / CONTRA ☐
AUSCULTADOR PILOT ☐
VIBRADOR B71 ☐
COLUNAS DE CAMPO LIVRE ☐

DIREITO ☐ ESQUERDO ☐

CALIBRAÇÃO EM SPL / HL ☐

FREQUÊNCIA	ATENUADOR	VALOR VERIFICADO	CORRECÇÃO	VALOR FINAL
125				
250				
500				
750				
1000				
1500				
2000				
3000				
4000				
6000				
8000				
10000				

ACESSÓRIOS VERIFICADOS

AUSCULTADORES TDH39 ☐
AUSCULTADORES ME70 ☐
AUSC. SENNHEISER HDA200 ☐
AUSC. IPSI / CONTRA ☐
AUSCULTADOR PILOT ☐
VIBRADOR B71 ☐
COLUNAS DE CAMPO LIVRE ☐

DIREITO ☐ ESQUERDO ☐

VERIFICAÇÃO EM SPL / HL ☐

FREQUÊNCIA	ATENUADOR	VALOR VERIFICADO	CORRECÇÃO	VALOR FINAL
125				
250				
500				
750				
1000				
1500				
2000				
3000				
4000				
6000				
8000				
10000				

ACESSÓRIOS VERIFICADOS

AUSCULTADORES TDH39 ☐
AUSCULTADORES ME70 ☐
AUSC. SENNHEISER HDA200 ☐
AUSC. IPSI / CONTRA ☐
AUSCULTADOR PILOT ☐
VIBRADOR B71 ☑
COLUNAS DE CAMPO LIVRE ☐

DIREITO ☑ ESQUERDO ☑

VERIFICAÇÃO EM SPL / HL ☐

FREQUÊNCIA	ATENUADOR	VALOR VERIFICADO	CORRECÇÃO	VALOR FINAL
125	—	—	—	—
250	25	83,2	- 8,9	74,8
500	40	90,8	- 10,1	80,7
750	40	77,8	- 6,2	71,6
1000	50	79,2	- 4,0	75,2
1500	50	71,8	- 2,9	68,9
2000	50	68,5	- 6,7	61,8
3000	50	68,1	- 5,9	62,2
4000	40	58,0	- 4,3	53,7
6000	40	65,6	- 8,2	57,4
8000				
10000				

Elaborado: GQ	Aprovado: DG	Revisão: 01 Data: Página 1 de 1

Modelo SGQ 44/00

8.4.2011

V.S.F.F.

GN Otometrics A/S CALIBRATION CERTIFICATE

Calibration chart for : B 71

Tone Norm : ISO 389-3 Instrument : OB922 Date : 10-Okt-2007
NBN Norm : Serial no : 277525 ID : WP

Freq Hz	Tone Norm	Mic/ Mast Corr.	Att. Pos.	Target Level *1/*2	Left output	Right output	NBN Norm	Target Level *3	Left output	Right output
125	NA			40	NA		4.00	40	NA	
250	67.00			40	39.6		4.00	40	39.9	
500	58.00			40	39.9		4.00	40	40.4	
750	48.50			40	40.2		5.00	40	39.7	
1000	42.50			40	39.9		6.00	40	39.5	
1500	36.50			40	39.6		6.00	40	40.0	
2000	31.00			40	40.0		6.00	40	39.8	
3000	30.00			40	39.6		6.00	40	39.7	
4000	35.50			40	39.8		5.00	40	39.9	
6000	40.00			40	39.9		5.00	40	39.9	
8000	40.00			40	NA		5.00	40	40.3	

Ext Norm :

Input	Ext Norm	Mic Corr. *7	Att. Pos.	Target Level *4/*5	Left output	Right output
WNoise	35.50			40	39.7	
SNoise	60.50			40	39.8	
CD1 *6/*8	57.50			40	39.9	
CD2 *6/*8	57.50			40	39.9	
Mic *6/*8	57.50			40	39.9	

Headband pressure :

Target	Value
5,4+/- 0,5N	5.5N

These remarks are not used in autocalibration mode.
*1 Tone Target level is norm + Mic. Correction + Attenuator Pos.(HL)
*2 Tone Target level is Mic. Correction + Attenuator Pos.(SPL)
*3 Noise Target level is Tone target level + noise norm.
*4 Target level is norm + Mic. Correction at 1000 Hz + Attenuator Pos.(HL)
*5 Target level is Mic. Correction at 1000 Hz + Attenuator Pos.(SPL)
*6 at @ 0 VU
*7 at @ 1000 Hz
*8 Use westra CD track 32 for CD1 and CD2 and track 49 for Mic.(ME Ge ISO)

7.7

noiselab
laboratório de engenheiros acústicos associados, lda

Medições de Ruído Ambiente

Nomeadamente:

- **Medição dos níveis de pressão sonora. Determinação do nível sonoro contínuo equivalente**

Seguindo os métodos de ensaios das normas e documentos:

NP ISO 1996-1: 2011, NP ISO 1996-2: 2011 e PT5:03/08/2018

Referência do Relatório: OF 992_19 RLEQ RIt01Vrs01

Cliente: Luís Roque dos Reis

Local do Ensaio:

Cabine Insonorizada localizada no
Serviço de Otorrinolaringologia do Hospital de Egas Moniz,
Centro Hospitalar Lisboa Ocidental

Datas das Medições: 11-03-2019

Data do Relatório: 19-06-2019

Total de Páginas: 6
(excluindo Anexos Externos identificados)

2. Resultados das Medições

Foram feitas medições no interior da câmara, a 1,5 m acima do pavimento da mesma, em várias situações – Ar condicionado ligado na velocidade máxima, na velocidade média e totalmente desligado. Os valores das várias medições, respetivos valores médios para cada regime de funcionamento da fonte de ruído existente no exterior da sala, e valores limites admissíveis referidos na Norma Americana ANSI S3.1-1999 - Maximum Permissible Ambient Noise Levels for Audiometric Test Rooms são apresentados de seguida:

Descrição	AC Ligado Vel. Max. Medição 1/3	AC Ligado Vel. Max. Medição 2/3	AC Ligado Vel. Max. Medição 3/3	AC Ligado Vel. Max. Valores Médio	Valores Máximos Admissíveis de ruído ambiente (MPANLs) - Norma ANSI S3.1-1999 - Table 3	Desvios relativos aos Valores Máximos Admissíveis de ruído ambiente (MPANLs) - Norma ANSI S3.1-1999 - Table 3
Nome da Medição	Projeto014	Projeto015	Projeto016			
Data e Hora de Início da Medição	12-03-2019 17:05	12-03-2019 17:06	12-03-2019 17:07			
Tempo de Medição	00:00:31	00:00:31	00:00:31			
Nível Contínuo Equivalente com Malha A Global - Leq [dBA]	18.2	18.3	18.3	18.2	-	
Níveis Leq [dB] por bandas de 1/3 de oit.						
LZeq 125Hz	0.4	0.7	1.9	1.0	24.0	-
LZeq 250Hz	-1.5	-1.5	-1.5	-1.5	16.0	-
LZeq 500Hz	-0.7	-0.7	-0.6	-0.7	11.0	-
LZeq 800Hz	0.4	0.3	0.3	0.3	10.0	-
LZeq 1kHz	1.1	1.0	1.0	1.0	8.0	-
LZeq 1.6kHz	2.7	2.7	2.6	2.7	9.0	-
LZeq 2kHz	3.6	3.6	3.6	3.6	9.0	-
LZeq 3.15kHz	5.6	5.6	5.6	5.6	8.0	-
LZeq 4kHz	6.6	6.6	6.6	6.6	6.0	0.6
LZeq 6.3kHz	8.6	8.6	8.6	8.6	8.0	0.6
LZeq 8kHz	9.6	9.8	9.8	9.7	9.0	0.7

Tabela 1- Resultados com Ar Condicionado da Sala de Apoio exterior à Câmara na Velocidade Máxima

Descrição	AC Ligado Vel. Média Medição 1/3	AC Ligado Vel. Média Medição 2/3	AC Ligado Vel. Média Medição 3/3	AC Ligado Vel. Média Valores Médio	Valores Máximos Admissíveis de ruído ambiente (MPANLs) - Norma ANSI S3.1-1999 - Table 3	Desvios relativos aos Valores Máximos Admissíveis de ruído ambiente (MPANLs) - Norma ANSI S3.1-1999 - Table 3
Nome da Medição	Projeto002	Projeto003	Projeto004			
Data e Hora de Início da Medição	12-03-2019 16:46	12-03-2019 16:48	12-03-2019 16:49			
Tempo de Medição	00:00:32	00:00:31	00:00:32			
Nível Contínuo Equivalente com Malha A Global - Leq [dBA]	18.3	18.3	18.3	18.3	-	
Níveis Leq [dB] por bandas de 1/3 de oit.						
LZeq 125Hz	2.3	1.0	1.0	1.5	24.0	-
LZeq 250Hz	-0.1	-1.4	-1.3	-0.9	16.0	-
LZeq 500Hz	-0.4	-0.4	-0.7	-0.5	11.0	-
LZeq 800Hz	0.4	0.5	0.4	0.4	10.0	-
LZeq 1kHz	1.2	1.1	1.1	1.1	8.0	-
LZeq 1.6kHz	2.7	2.8	2.7	2.7	9.0	-
LZeq 2kHz	3.6	3.7	3.7	3.7	9.0	-
LZeq 3.15kHz	5.6	5.6	5.6	5.6	8.0	-
LZeq 4kHz	6.8	6.8	6.8	6.8	6.0	0.8
LZeq 6.3kHz	8.7	8.7	8.7	8.7	8.0	0.7
LZeq 8kHz	9.7	9.7	9.7	9.7	9.0	0.7

Tabela 2- Resultados com Ar Condicionado da Sala de Apoio exterior à Câmara na Velocidade Média

Descrição	AC Desligado Medição 1/3	AC Desligado Medição 2/3	AC Desligado Medição 3/3	AC Desligado Valores Médio	Valores Máximos Admissíveis de ruído ambiente (MPANLs) - Norma ANSI S3.1-1999 - Table 3	Desvios relativos aos Valores Máximos Admissíveis de ruído ambiente (MPANLs) - Norma ANSI S3.1-1999 - Table 3
Nome da Medição	Projeto017	Projeto018	Projeto019			
Data e Hora de Início da Medição	12-03-2019 17:08	12-03-2019 17:09	12-03-2019 17:10			
Tempo de Medição	00:00:31	00:00:31	00:00:31			
Nível Contínuo Equivalente com Malha A Global - Leq [dBA]	18.2	18.3	18.2	18.2	-	
Níveis Leq [dB] por bandas de 1/3 de oit.						
LZeq 125Hz	1.1	0.7	1.4	1.0	24.0	-
LZeq 250Hz	-1.5	-1.5	-1.5	-1.5	16.0	-
LZeq 500Hz	-0.6	-0.7	-0.7	-0.6	11.0	-
LZeq 800Hz	0.3	0.3	0.2	0.3	10.0	-
LZeq 1kHz	1.0	1.0	1.0	1.0	8.0	-
LZeq 1.6kHz	2.7	2.7	2.7	2.7	9.0	-
LZeq 2kHz	3.6	3.6	3.6	3.6	9.0	-
LZeq 3.15kHz	5.6	5.7	5.6	5.6	8.0	-
LZeq 4kHz	6.7	6.6	6.6	6.6	6.0	0.6
LZeq 6.3kHz	8.7	8.7	8.6	8.6	8.0	0.6
LZeq 8kHz	9.6	9.9	9.6	9.7	9.0	0.7

Tabela 3- Resultados com Ar Condicionado da Sala de Apoio exterior à Câmara, Desligado

3. Conclusões

O ruído do ar condicionado existente na sala onde se encontra a cabine não teve influência nos níveis de ruído medidos no interior da mesma. Na grande maioria das bandas de frequência, os valores medidos encontram-se abaixo dos valores máximos admissíveis descritos na tabela 3 da Norma Norma ANSI S3.1-1999. Nos casos em que se encontra acima é por valores reduzidos, entre 0.6 e 0.8 dB.

Sarilhos Pequenos, 19 de Junho de 2019

Medição e Relatório efectuados por:

Paulo Valério – Engº Físico

Validity unknown

Digitally signed by LABMETRO ONLINE
Date: 2018.01.23 10:28:46 +00:00
Reason: Documento aprovado electronicamente

CERTIFICADO DE VERIFICAÇÃO

NÚMERO 245.70 / 18.048065

ENTIDADE:

Nome	Noise Lab - Laboratório de Engenheiros Acústicos Associados, Lda.
Endereço	Rua dos Salineiros, nº 7 - Moita - 2860-642 Sarilhos Pequenos

INSTRUMENTO DE MEDIÇÃO:

Desp. Aprov. Modelo n.º	245.70.08.3.14	
Sonómetro	Marca / Modelo / Nº de série / Selo Nº	Brüel & Kjær / 2270 / 2644650 / 048065
Microfone	Marca / Modelo / Nº de série	Brüel & Kjær / 4189 / 2643574
Pré-amplificador	Marca / Modelo / Nº de série	Brüel & Kjær / ZC 0032 / 9591
Calibrador	Marca / Modelo / Nº de série / Selo Nº	Brüel & Kjær / 4231 / 2651797 / 048065

CARACTERÍSTICAS METROLÓGICAS:

Classe	1

OPERAÇÃO EFECTUADA:

Tipo / Data	Verificação Periódica / 18/01/2018
Rastreabilidade	Tensão contínua e alternada - Lab. Metrol. Eléct. ISQ (Portugal) Frequência - IPQ (Portugal) Nível de pressão sonora - Danak (Dinamarca)
Documentos de referência	Portaria 977/09 de 1 de Setembro de 2009 Proc. Interno PO.M-DM/ACUS 02 (Ed. C - Rev. 00) tendo por base os documentos de referência Norma IEC 61672-3: 2006-10
Condições ambientais	Temp.: 23,1 ºC Hum. Rel.: 49,0 % Pressão atmosf.: 100,3 kPa
RESULTADO	**Em conformidade com os valores regulamentares** **O Valor do erro de cada uma das medições efectuadas são inferiores aos valores dos erros máximos admissíveis para a classe do equipamento de medição**

Local / Data	Verificado por	Responsável pela Validação
Oeiras, 18 de janeiro de 2018	Ana Colaço	Luís Ferreira (Responsável Técnico)

O presente Boletim de Verificação só pode ser reproduzido no seu todo e apenas se refere ao(s) item(s) ensaiado(s).
O equipamento é selado como consta no Despacho de aprovação de modelo respectivo.
A operação de controlo metrológico efectuada é evidenciada apenas pela aposição no instrumento do símbolo respectivo como consta dos anexos da Portaria n.º 962/90 de 9 de Setembro

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7.8

Table 21- 20-30 years, results in hearing thresholds (dB) depending on the frequencies (Hz), in occluded and unoccluded conditions.

Age	**Ear**		**250 Hz**			**500 Hz**			**1000 Hz**			**2000 Hz**			**Aver.**	
(years)		no	occ	diff	no	occ	diff	no	occ	diff	no	occ	diff	no	occ	diff
	R	5	25	**20**	10	30	**20**	15	40	**25**	10	40	**30**	10	33,8	23,8
	L	5	10	**5**	10	25	**15**	5	35	**30**	5	35	**30**	6,3	26,3	20
	R	5	30	**25**	10	35	**25**	5	30	**25**	10	40	**30**	7,5	33,8	26,3
	L	5	25	**20**	5	35	**30**	5	30	**25**	0	30	**30**	3,8	30	26,3
	R	0	15	**15**	10	30	**20**	5	35	**30**	5	45	**40**	5	31,3	26,3
	L	0	20	**20**	15	30	**15**	0	20	**20**	5	40	**35**	5	27,5	22,5
	R	5	15	**10**	5	25	**20**	5	30	**25**	10	40	**30**	6,3	27,5	21,3
	L	5	20	**15**	0	30	**30**	0	25	**25**	0	40	**40**	1,3	28,8	27,5
	R	10	15	**5**	10	25	**15**	10	35	**25**	15	35	**20**	11,3	27,5	16,3
	L	15	15	**0**	10	30	**20**	5	30	**25**	5	40	**35**	8,8	28,8	20
	R	15	30	**15**	15	25	**10**	15	35	**20**	20	50	**30**	16,3	35	18,8
	L	10	20	**10**	15	30	**15**	10	35	**25**	10	45	**35**	11,3	32,5	21,3
	R	10	35	**25**	10	40	**30**	5	35	**30**	10	40	**30**	8,8	37,5	28,8
	L	20	25	**5**	10	40	**30**	10	45	**35**	10	40	**30**	12,5	37,5	25
	R	5	20	**15**	5	35	**30**	10	40	**30**	5	40	**35**	6,3	33,8	27,5
	L	0	15	**15**	5	20	**15**	5	25	**20**	0	30	**30**	2,5	22,5	20
	R	10	25	**15**	0	25	**25**	10	45	**35**	10	45	**35**	7,5	35	27,5
	L	10	20	**10**	5	25	**20**	0	30	**30**	0	35	**35**	3,8	27,5	23,8
	R	5	25	**20**	10	30	**20**	10	40	**30**	0	40	**40**	6,3	33,8	27,5
	L	15	20	**5**	10	25	**15**	5	25	**20**	0	35	**35**	7,5	26,3	18,8
	R	5	10	**5**	10	30	**20**	10	35	**25**	10	50	**40**	8,8	31,3	22,5
	L	10	10	**0**	5	30	**25**	5	25	**20**	10	40	**30**	7,5	26,3	18,8
	R	15	20	**5**	10	30	**20**	10	35	**25**	10	40	**30**	11,3	31,3	20
	L	10	10	**0**	10	20	**10**	0	25	**25**	5	35	**30**	6,3	22,5	16,3
	R	20	25	**5**	20	35	**15**	20	50	**30**	20	50	**30**	20	40	20
	L	10	10	**0**	20	20	**0**	5	30	**25**	0	35	**35**	8,8	23,8	15
	R	5	5	**0**	0	20	**20**	5	25	**20**	10	35	**25**	5	21,3	16,3
	L	0	5	**5**	5	15	**10**	0	25	**25**	0	30	**30**	1,3	18,8	17,5
	R	10	30	**20**	5	35	**30**	10	35	**25**	10	45	**35**	8,8	36,3	27,5
	L	10	30	**20**	5	35	**30**	10	35	**25**	10	45	**35**	8,8	36,3	27,5
	R	10	30	**20**	5	20	**15**	10	35	**25**	15	50	**35**	10	33,8	23,8
	L	10	20	**10**	5	20	**15**	0	25	**25**	5	25	**20**	5	22,5	17,5
	R	15	25	**10**	5	25	**20**	0	25	**25**	0	35	**35**	5	27,5	22,5
	L	20	35	**15**	10	30	**20**	5	30	**25**	10	35	**25**	11,3	32,5	21,3
	R	15	30	**15**	0	20	**20**	5	30	**25**	5	40	**35**	6,3	30	23,8
	L	10	15	**5**	0	15	**15**	0	35	**35**	0	40	**40**	2,5	26,3	23,8
	R	10	30	**20**	5	20	**15**	10	40	**30**	5	40	**35**	7,5	32,5	25
	L	10	10	**0**	10	20	**10**	0	25	**25**	5	30	**25**	6,3	21,3	15
	R	15	25	**10**	5	30	**25**	15	35	**20**	20	45	**25**	13,8	33,8	20
	L	5	15	**10**	0	20	**20**	10	40	**30**	5	35	**30**	5	27,5	22,5
25.6	-	9.3	20.4	**11.1**	7,6	27	**19.4**	6,6	32,6	**26**	7,1	39,1	**32**	**7,7**	**29,8**	**22,1**

R: right; L: left; no: without occlusion; occ: with occlusion; diff: difference; Aver.: average

Table 22- 40-50 years, results in hearing thresholds (dB) depending on the frequencies (Hz), in occluded and unoccluded conditions.

Age	Ear	250 Hz			500 Hz			1000 Hz			2000 Hz			Aver.		
(years)		no	occ	diff	no	occ	diff	no	occ	diff	no	occ	diff	no	occ	diff
42	R	10	40	**30**	5	30	**25**	5	25	**20**	5	35	**30**	6,3	32,5	26,3
	L	10	35	**25**	5	30	**25**	5	40	**35**	10	40	**30**	7,5	36,3	28,8
47	R	20	25	**5**	10	25	**15**	10	25	**15**	10	30	**20**	12,5	26,3	13,8
	L	20	45	**25**	15	40	**25**	10	30	**20**	5	35	**30**	12,5	37,5	25
46	R	10	20	**10**	10	15	**5**	15	25	**10**	5	20	**15**	10	20	10
	L	10	30	**20**	15	20	**5**	15	30	**15**	15	35	**20**	13,8	28,8	15
49	R	15	35	**20**	10	25	**15**	5	30	**25**	5	40	**35**	8,8	32,5	23,8
	L	20	50	**30**	15	40	**25**	5	35	**30**	10	40	**30**	12,5	41,3	28,8
50	R	10	40	**30**	5	30	**25**	5	15	**10**	10	45	**35**	7,5	32,5	25
	L	10	35	**25**	10	30	**20**	5	25	**20**	5	35	**30**	7,5	31,3	23,8
50	R	15	40	**25**	10	40	**30**	10	30	**20**	5	35	**30**	10	36,3	26,3
	L	10	25	**15**	10	20	**10**	10	25	**15**	5	25	**20**	8,8	23,8	15
41	R	15	25	**10**	15	30	**15**	10	35	**25**	10	30	**20**	12,5	30	17,5
	L	15	30	**15**	10	40	**30**	5	35	**30**	15	35	**20**	11,3	35	23,8
41	R	10	25	**15**	10	30	**20**	10	30	**20**	10	35	**25**	10	30	20
	L	5	15	**10**	10	30	**20**	15	35	**20**	20	40	**20**	12,5	30	17,5
41	R	15	25	**10**	15	35	**20**	0	25	**25**	0	30	**30**	7,5	28,8	21,3
	L	10	20	**10**	5	25	**20**	0	25	**25**	0	30	**30**	3,8	25	21,3
47	R	15	30	**15**	5	25	**20**	5	30	**25**	0	30	**30**	6,3	28,8	22,5
	L	15	25	**10**	10	30	**20**	10	35	**25**	15	40	**25**	12,5	32,5	20
43	R	10	30	**20**	5	15	**10**	10	25	**15**	0	25	**25**	6,3	23,8	17,5
	L	10	15	**5**	10	20	**10**	10	25	**15**	20	40	**20**	12,5	25	12,5
45.2	-	12.7	30	**17.3**	9.8	28.4	**18.6**	8	28.9	**20.9**	8.2	34.1	**25.9**	**9,7**	**30,4**	**20.7**

R: right; L: left; no: without occlusion; occ: with occlusion; diff: difference; Aver.: average

Table 23- 60-70 years, results in hearing thresholds (dB) depending on the frequencies (Hz), in occluded and unoccluded conditions.

Age	**Ear**		**250 Hz**			**500 Hz**			**1000 Hz**			**2000 Hz**			**Aver.**	
(years)		no	occ	diff	no	occ	diff	no	occ	diff	no	occ	diff	no	occ	diff
62	R	25	45	**20**	20	40	**20**	20	25	**5**	25	45	**20**	22,5	38,8	16,3
	L	20	50	**30**	15	45	**30**	15	35	**20**	25	55	**30**	18,8	46,3	27,5
60	R	20	45	**25**	15	40	**25**	10	35	**25**	15	40	**25**	15	40	25
	L	25	45	**20**	15	40	**25**	15	35	**20**	20	45	**25**	18,8	41,3	22,5
67	R	25	35	**10**	20	40	**20**	20	40	**20**	15	45	**30**	20	40	20
	L	10	35	**25**	15	30	**15**	10	40	**30**	5	40	**35**	10	36,3	26,3
62	R	15	35	**20**	10	30	**20**	10	35	**25**	30	50	**20**	16,3	37,5	21,3
	L	20	50	**30**	15	40	**25**	5	35	**30**	30	60	**30**	17,5	46,3	28,8
65	R	30	45	**15**	30	55	**25**	25	50	**25**	20	60	**40**	26,3	52,5	26,3
	L	35	40	**5**	30	45	**15**	25	55	**30**	20	70	**50**	27,5	52,5	25
60	R	20	30	**10**	10	25	**15**	5	35	**30**	10	45	**35**	11,3	33,8	22,5
	L	15	20	**5**	15	25	**10**	10	30	**20**	30	40	**10**	17,5	28,8	11,3
63	R	20	35	**15**	10	25	**15**	10	30	**20**	15	40	**25**	13,8	32,5	18,8
	L	15	20	**5**	15	35	**20**	15	45	**30**	20	55	**35**	16,3	38,8	22,5
66	R	15	40	**25**	10	35	**25**	5	35	**30**	20	55	**35**	12,5	41,3	28,8
	L	25	30	**5**	25	30	**5**	35	45	**10**	40	65	**25**	31,3	42,5	11,3
63	R	10	35	**25**	15	25	**10**	10	35	**25**	10	35	**25**	11,3	32,5	21,3
	L	20	25	**5**	15	30	**15**	15	40	**25**	20	45	**25**	17,5	35	17,5
62	R	10	25	**15**	10	30	**20**	0	20	**20**	0	30	**30**	5	26,3	21,3
	L	10	30	**20**	10	30	**20**	10	40	**30**	20	40	**20**	12,5	35	22,5
63	-	19.3	35.8	**16.5**	16	34.8	**18.8**	13.5	37	**23.5**	19.5	48	**28.5**	**17,1**	**38,9**	**21,8**

R: right; L: left; no: without occlusion; occ: with occlusion; diff: difference; Aver.: average.

Table 24- Results of pure tone audiometry in the affected ear (right or left) and comparison with the results of the COT.

	Age	Pure tone audiometry (bc/ac, dB HL)						ABG	PTA	gap	gap	Weber test*					COT*				
		125 Hz	250 Hz	500 Hz	1000 Hz	2000 Hz	4000 Hz	(dB HL)	(dB HL)	250 Hz	500 Hz	128 Hz	256 Hz	512 Hz	1024 Hz	2048 Hz	128 Hz	256 Hz	512 Hz	1024 Hz	2048 Hz
1	67	-	0	10	15	25	20	12.5	32.5	35	25	A	A	A	A	I	I	I	I	I	I
		30	35	35	30	30	25														
2	50	-	15	20	20	25	15	37.5	60	45	40	A	A	A	A	I	A	A	A	A	A
		70	60	60	55	65	50														
3	68	-	10	10	15	10	25	23.75	42.5	45	35	A	A	A	A	A	A	A	A	A	I
		55	55	45	40	30	40														
4	24	-	10	10	5	15	10	25	33.75	25	20	A	A	A	A	I	A	A	A	NA	NA
		-	35	30	35	35	40														
5	12	-	5	5	5	5	5	19.25	25	35	20	A	A	A	A	A	I	I	I	A	A
		40	40	25	15	20	35														
6	58	-	15	20	20	25	65	23.75	50	40	35	A	A	A	A	I	A	A	A	A	I
		50	55	55	45	45	80														
7	41	-	10	15	20	30	30	28.75	50	35	40	A	A	A	A	I	A	A	A	I	I
		40	45	55	55	45	55														
8	55	-	20	20	5	25	40	23.75	40	30	25	A	A	A	A	A	A	A	A	A	A
		55	50	45	30	35	65														
9	32	-	10	5	15	25	10	27.5	43.75	30	40	A	A	A	A	A	A	NA	NA	A	A
		-	40	45	45	45	30														
10	68	-	5	10	15	10	10	16.25	32.5	35	30	A	A	A	A	I	I	I	I	I	I
		45	40	40	25	25	10														
11	49	-	25	35	35	50	45	25	67.5	45	40	A	A	A	A	A	NA	I	A	I	I
		75	70	75	65	60	65														
12	67	-	15	15	15	25	45	40	63.75	50	50	A	A	A	A	A	NA	A	A	I	NA
		50	60	65	65	65	65														
13	41	-	-10	0	5	5	0	6.25	8.75	15	5	A	A	A	A	A	I	I	I	NA	NA
		20	5	5	15	10	5														
14	14	-	5	5	0	5	5	20	22.5	25	15	I	I	A	A	A	I	I	I	A	A
		-	30	20	20	20	35														
15	61	-	5	15	25	30	55	18.75	43.75	45	35	I	I	A	I	I	I	I	A	I	I
		50	50	50	40	35	75														
16	23	-	15	10	15	20	35	12.5	37.5	40	25	A	A	A	A	A	A	I	A	A	A
		65	55	35	35	25	35														
17	50	-	5	10	10	15	20	10	21.25	15	10	A	A	A	A	A	A	A	A	A	A
		25	20	20	20	25	30														
18	47	-	-	10	20	10	5	22.5	43.75		40	A	A	A	I	I	I	A	A	I	I
		-	55	50	45	25	15														
19	53	-	10	15	20	25	30	31,25	40	35	30	A	A	A	A	I	A	I	A	I	I
		50	45	45	50	30	55														
20	60	-	20	30	25	30	20	31,25	65	55	40	A	A	A	A	A	A	A	A	A	A
		65	75	70	65	50	45														
21	57	-	5	10	25	30	20	13.75	40	45	30	A	A	A	A	I	A	A	A	A	I
		50	50	40	40	30	20														
22	50	-	15	20	15	30	25	41,24	63.75	50	45	A	A	A	A	A	A	A	A	A	A
		70	65	65	70	55	65														
23	56	-	15	20	10	20	30	20	37.5	30	15	A	A	A	I	I	A	A	I	I	I
		50	45	35	30	40	55														
24	38	-	25	35	25	35	30	33.75	66.25	45	30	A	A	A	A	A	A	A	A	A	I
		70	65	65	65	70	60														
25	50*	-	10	20	30	55	50	25	66.25	55	40	A	A	A	A	A	A	A	A	A	A
		75	65	60	70	70	55														
26	59	-	5	15	15	10	15	33.75	52.5	50	45	A	A	A	A	A	A	A	A	A	A
		55	55	60	60	35	35														
27	59	-	5	20	15	20	25	17.5	41.25	45	30	A	A	A	A	A	A	A	I	I	I
		50	50	50	35	30	35														
28	13	-	5	15	5	15	5	18.75	33.75	40	25	A	A	A	A	A	NA	A	NA	NA	NA
		50	45	40	25	25	25														
29	45	-	5	0	0	15	10	12.5	20	15	15	A	A	A	I	I	NA	NA	I	I	I
		-	20	15	20	25	25														
30	40	-	10	20	30	40	35	23.75	58.75	50	35	A	A	A	A	I	A	NA	A	A	A
		55	60	55	60	60	45														
31	52	-	20	15	25	10	20	36.25	56.25	45	45	A	A	A	A	I	A	A	A	A	I
		50	65	60	65	35	55														
32	21*	-	25	20	20	30	20	32.5	56.25	35	40	A	A	A	A	A	A	A	A	I	A
		-	60	60	55	50	55														
33	22	-	10	15	10	10	5	43.75	55	45	35	A	A	A	A	A	I	A	A	A	A
		50	55	60	55	50	50														
34	61*	-	10	10	10	15	10	5	18.75	30	10	I	I	A	NA	NA	NA	NA	I	NA	NA
		15	20	20	20	15	10														
35	46	-	10	5	15	15	40	30	41.25	40	35	A	A	A	A	A	A	A	A	A	I
		55	50	40	35	40	80														
36	71	-	40	55	45	55	45	36.25	88.75	10	15	A	A	A	A	I	A	A	A	A	I
		-	95	95	85	80	85														
37	41	-	5	10	5	15	15	40	51.25	40	45	A	A	A	A	A	A	A	A	A	A
		30	45	65	40	55	45														
38	32	-	20	20	15	30	20	48.75	71.25	45	50	A	A	A	A	A	I	A	A	I	A
		65	65	70	80	70	60														
39	48	-	0	10	5	10	15	13.75	26.25	25	20	A	A	A	I	I	I	A	I	I	I
		25	25	30	30	20	15														
40	42	-	0	0	10	15	20	11.25	22.5	25	25	A	A	A	I	I	I	I	I	I	I
		25	25	25	20	20	25														
41	42	-	15	20	20	25	30	12.5	40	30	10	A	A	A	A	A	I	I	I	I	A
		45	45	30	45	40	30														
42	61	-	5	20	15	25	30	22.5	47.5	50	35	A	A	A	A	I	A	A	A	A	A
		50	55	55	45	35	45														
43	69	-	10	25	15	20	20	23.75	46.25	45	30	A	A	A	A	A	NA	A	A	A	A
		-	55	55	35	40	45														
44	60	-	15	25	40	55	45	36.25	66.25	35	30	I	A	A	I	I	I	I	A	A	I
		55	50	55	75	85	95														
45	50	-	10	15	30	60	65	36.25	71.25	55	55	A	I	A	A	I	I	I	A	I	NA
		55	65	70	70	80	95														
46	79	-	5	5	30	40	50	40	71.25	70	75	I	A	A	A	A	I	I	A	A	I
		80	75	80	70	60	75														
47	55	-	5	10	10	15	10	12.5	23.75	20	15	A	A	A	I	A	NA	NA	NA	NA	NA
		20	25	25	20	25	25														
48	69	-	15	30	20	5	20	16.25	41.25	45	25	A	A	A	A	I	A	A	A	A	I
		50	60	55	35	15	25														
49	46	-	5	0	0	5	30	21.25	23.75	20	30	I	A	A	I	I	I	I	I	I	I
		20	25	30	25	15	45														
50	42	-	0	0	5	10	25	38.75	48.75	50	60	A	A	A	A	A	I	A	A	A	I
		45	50	60	50	35	50														
51	54	-	30	40	40	35	40	35	73.75	50	40	A	A	A	I	I	I	A	A	I	I
		85	80	80	75	60	80														
52	39	-	15	25	15	25	20	32.5	50	25	25	A	A	A	A	A	A	A	A	A	A
		45	40	50	45	65	55														
53	16	-	0	5	0	0	5	50	46.25	40	40	I	A	A	A	A	I	I	A	NA	NA
		30	40	45	40	60	65														
								29,3	44,6	38,1	32,1										

bc: bone-conduction thresholds; ac: air-conduction tl
* tuning fork frequencies.
ABG: 0.5, 1, 2, and 4 kHz
PTA: arithmetic average of 4 consecutive frequencies 250 Hz, 500 Hz, 1000 Hz and 2000 Hz

7.9

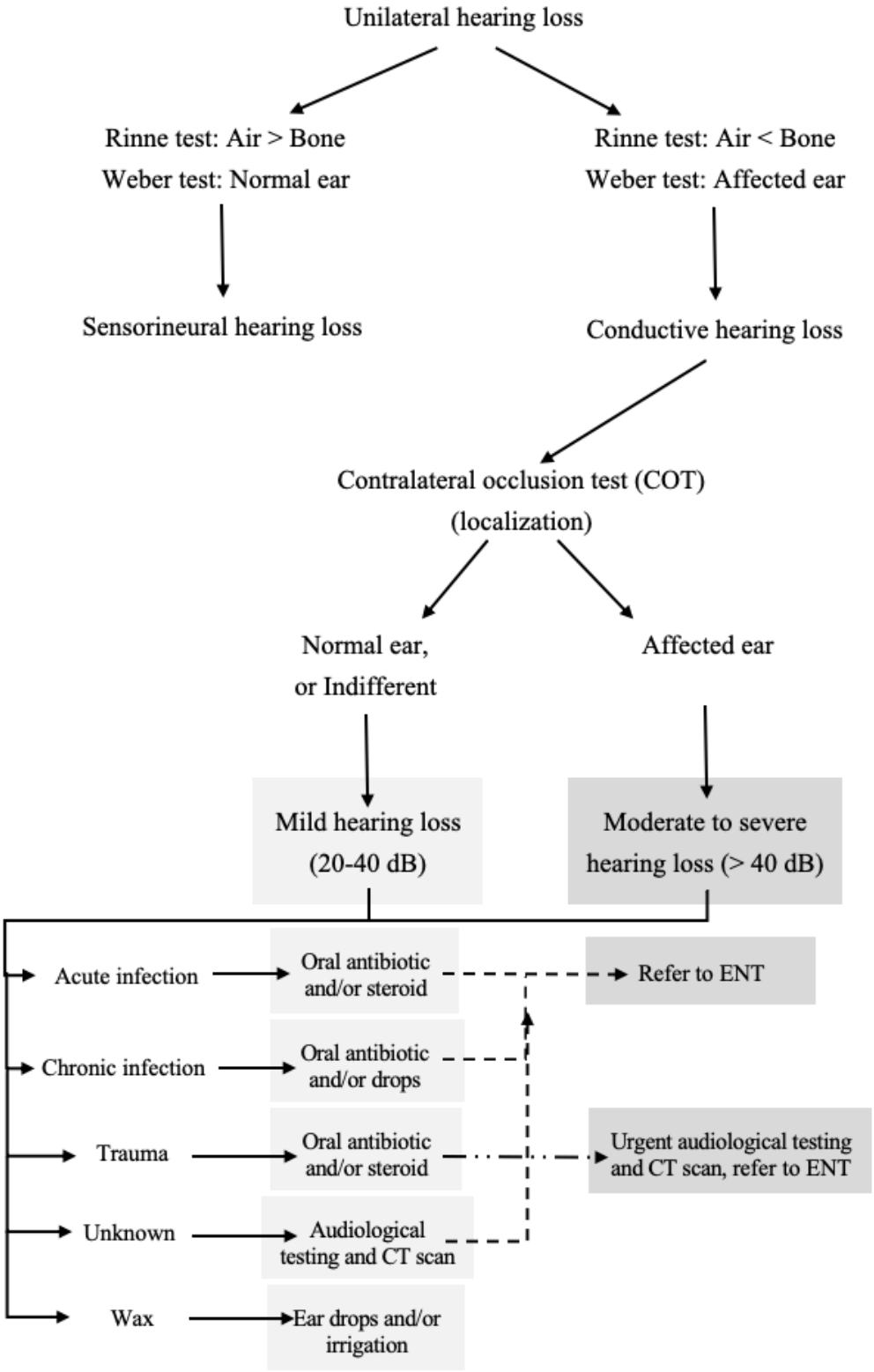

Figure 24- Flowchart of contralateral occlusion test (COT): how to manage hearing loss?